Arylhydrocarbon receptor activation promotes epidermal barrier formation and attenuates Th2-cytokine dependent inflammatory processes: The biological basis of coal tar therapy in atopic dermatitis

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Coal tar therapy is the oldest treatment for atopic dermatitis (AD), a T helper 2 (Th2) mediated skin disease characterized by epidermal barrier dysfunction associated with filaggrin loss-of-function mutations. However, due to cosmetic aspects, suspected -albeit unproven- carcinogenicity and an hitherto unknown molecular mechanism, coal tar therapy is progressively abandoned by dermatologists. The effect of coal tar on submerged and 3D cultured human keratinocytes, and in lesional AD skin were studied by genetic (siRNA) and cell biological (gene/protein expression, protein phosphorylation) analysis. Here we found coal tar to activate the arylhydrocarbon receptor (AhR) signaling pathway, thereby inducing epidermal differentiation gene and protein expression resulting in accelerated epidermal differentiation. AhR knockdown abolished the coal tar-mediated induction of major epidermal barrier proteins, including filaggrin. Coal tar restored filaggrin haploinsufficiency and Th2 cytokine-mediated downregulation of terminal differentiation proteins. Furthermore, coal tar reduced spongiosis and apoptosis, and downregulated the expression of eosinophilic chemoattractant CCL26, all induced by Th2 cytokine signaling. These effects were mediated through AhR-regulated STAT6 dephosphorylation, via activation of the nuclear factor-erytroid 2-related factor-2 (Nrf2) anti-oxidative stress pathway. This study not only provides a brand new perspective to the oldest known drug in dermatological practice, it might also open a new avenue for pharmaceutical industries to reevaluate the AhR as a bona fide pharmacological target thereby enabling the development of mechanism-based drugs for AD.

IL-1beta as a therapeutic target in Epidermolysis bullosa simplex

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Epidermolysis bullosa simplex type Dowling-Meara (EBS-DM) is a severe subtype of EBS which is frequently caused by a heterozygous mutation in position 435 of the keratin 14 (K14) gene. This dominant negative acting mutation leads to a self-aggregation of K14 filaments in the periphery of the cytoplasm and to a subsequent collapse of the intermediate filament network, resulting in the characteristic blistering and erosions of the skin after minor trauma. Expression profiling showed that the inflammatory cytokine IL-1beta was significantly upregulated in K14 mutated patient keratinocyte cell lines. Increased K14 expression levels were accompanied by a constitutive activation of the JNK/MAPK-stress pathway. Here we show that EBS-DM specific overexpression of K14 protein and its subsequent aggregation was linked to increased levels of mature IL-1beta, which in turn activated the JNK/MAPK pathway. This activation again led to increased expression levels of K14, ending up in a positive feedback loop. We achieved a downregulation of K14 expression levels and JNK phosphorylation by treatment with a neutralizing IL-1beta antibody and the IL-1beta inhibiting small molecule diacerein. These preclinical data have formed the basis of a clinical pilot trial in EBS-DM patients, which is currently ongoing.

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Epidermal ablation of EGFR impairs skin immuno-homeostasis and epithelial differentiation: implication in the pathogenesis of the rash in patients treated with anti EGFR drugs

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The clinical use of several anti-EGFR agents revealed common adverse effects targeting skin and skin adnexa. To gain insight into the etiology of these adverse effects, we developed an epidermally targeted mouse model of EGFR ablation. EGFR was ablated in the epidermis in the litters derived from Keratin5-driven Cre-Recombinase transgenics, crossed with EGFR floxed mice. The skin of double transgenic mice reproduced the hallmarks of the skin lesions of patients undergoing chemotherapy with anti-EGFR agents: infiltration of macrophages, mast cells, and lymphocytes followed by formation of neutrophilic pustules. Epidermally ablated EGFR mice showed highly pruritic and scaly skin with formation of keratin plugs and hair follicle destruction. Tissue samples isolated from skin of double transgenics contained high mRNA levels of several inflammatory mediators. Attempts to ameliorate or rescue the inflammatory phenotype by crossing double transgenics with knockout mice for TNFR1/2, MyD-88, iNOS, CCR2 and Rag1 were unsuccessful indicating that TNF-α, IL-1 family members, iNOS, CCL2 and lymphocytes were not primary drivers of the full inflammatory phenotype. Immuno-histochemical analysis of skin cells isolated prior to the development of the inflammatory phenotype showed enhanced F4/80 macrophage infiltration. Subcutaneous injection of clodronate liposomes into double transgenic mice reduced skin infiltrating macrophages and mast cells, decreased overall inflammation, and lowered the expression of several inflammatory mediators. Moreover, clodronate administration partially restored skin architecture and differentiation by decreasing the expression of epidermal differentiation markers. Thus aberrant macrophage recruitment appears to have a primary role in the development of the lesions in the EGFR ablated skin and can interfere with a proper differentiation program. These data highlight the master role of epidermal EGFR in shaping immune cell driven inflammation and differentiation in the skin.

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Combination of PLS3, Twist, CD158k/KIR3DL2 and NKp46 gene expression for the diagnosis of Sezary Syndrome

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Several molecular markers including T-plastin (PLS3), transcription factor Twist, CD158k/KIR3DL2 and NKp46 (CD335) have been specifically identified in patients with Sézary Syndrome (SS), the erythrodermic and leukemic form of cutaneous T-cell lymphoma (CTCL). Our purpose was to investigate whether the expression profiling of these four genes by quantitative Real-time PCR (qRT-PCR) can be employed for the diagnosis of SS. A cohort of 81 patients with SS was investigated for tumor burden and mRNA expression quantification of PLS3, Twist, KIR3DL2, and NKp46, in CD4+-purified T cells from blood samples using SYBR Green qPCR and specific primer pairs. CD4+-purified T cells from 12 healthy donors were studied as controls, with qRT-PCR mean values (±SD) of PLS3, Twist, KIR3DL2, and NKp46 mRNA levels reaching 2.4±2.5, 6.6±8, 22.5±20.4, and 2.6±2.8, respectively. A respective threshold of 95% significance was set up at 5, 10, 25 and 50, and any value less than the respective threshold was considered as negative. As positive controls we used mRNA mean levels (±SD) detected in SS HuT-78 cell line cultures (n=3) with 128±65 for PLS3, 13155±3000 for Twist, 316±40 for KIR3DL2, and 7.3±1.9 NKp46, respectively, as mRNA mean levels (±SD) in NK-purified cells (n=3) reaching 0.4±0.1, 0.5±0.15, 684±80.3, and 371±80.2, respectively. Our results demonstrated that qRT-PCR data accurately classified 100% of 81 SS patients with high blood tumor burden. CD4+-purified T cells from SS patients expressed PLS3, Twist, KIR3DL2, and NKp46 mRNA mean levels (±SEM) of 706±133, 1440±292, 843±96, and 7.1±1.7, respectively. The accuracy was 100% in identifying these samples as SS patients since the four markers were detected in 20% CD4+-purified T cell samples, three ones in 53%, two ones 20% and only one marker (Twist, PLS3 or Nkp46) in 7%. These results clearly demonstrate that gene expression profiling by quantitative PCR on a selected number of 4 critical genes can be employed for the molecular diagnostic of SS.

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Multicolor luciferase reporter gene assay system for IL-1β, 2, 8 and IFN-γ presents a novel tool to evaluate immunological effects of drugs and their efficacy

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Various immunosuppressant drugs are widely used against immune-mediated skin diseases. However, the relative potency of each drug and the mechanism how to suppress immune response are mostly unclear. In particular, it is difficult to determine whether each drug induces immunological effects through its effects on T cells or antigen presenting cells. So, we established a luciferase reporter gene assay system that can easily and briefly evaluate the effects of chemicals on T cells and monocytes (Mos), respectively (Multi-ImmunoTox Assay; MITA). The system is composed of the following two stable cell lines. #2H4 derived from Jurkat T cells is transfected with the vectors containing SLG luciferase regulated by IL-2 promoter, SLO luciferase by IFN-γ promoter, and SLR luciferase by G3PDH promoter. #142-7 derived from THP-1 monocytes is transfected with those containing SLG regulated by IL-1β promoter, SLO by IL-8 promoter, and SLR by G3PDH promoter. We evaluated various immunosuppressant drugs by this system. The results corresponded well to the previously reported immunological effects of the following drugs on T cells and Mos. Namely cyclosporin A (CyA) and tacrolimus (TRL) dramatically reduced IL-2 and IFN-γ transcription by #2H4, and significantly but weakly IL-8 transcription, or none of IL-1β transcription by #142-7. The potency of TRL was ten times higher than that of CyA. On the other hand, dexamethasone significantly suppressed the transcription of all 4 cytokines, although its effects on T cell cytokine was less potent than those of CyA. Finally, we succeeded in demonstrating that two sulfonamide, diaminodiphenyl sulfone (DDS) and sulfasalazine, whose immunological action is yet to be determined, significantly suppressed the cytokine transcription by #2H4 and #142-7, and that sulfasalazine was more potent than DDS. These data suggest that MITA can present a novel high-throughput approach to evaluate immunological effects of various drugs and their efficacy.

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Novel rapid diagnostic tests in the management of severe drug hypersensitivity

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Drug hypersensitivity reactions (DHR) are common and occasionally fatal. Dermatologists are often the first to identify these potentially severe systemic reactions because of the early involvement of the skin. Rapid intervention can halt or limit the progression of such immunologically mediated reactions but usually involves cessation of all medications. Lymphocyte proliferation (LPA) is the most widely utilised assay of drug hypersensitivity but is not routinely available. To investigate the usefulness of ex vivo testing for determination of the culprit drug during acute DHR we compared the LPA with an overnight assay for drug-specific IFN-γ and IL-4 release in 23 cases during an acute reaction, 31 patients post-recovery and 14 control subjects without DHR. For each patient up to five possible culprit drugs were identified from the ingestion chart by an experienced clinician. Reactions to 61 drugs in total were examined, including antibiotics (60%) and anticonvulsants (16%). All individuals clinically improved when the suspected culprit drugs were stopped. Healthy controls showed negative drug-specific proliferation and cytokine release in contrast to individuals with a confirmed sensitivity ($p<0.0001$) and the assays demonstrated a test specificity of 95.11% (LPA), 82.85% (IFN-γ) and 92% (IL-4). Combined measurement of drug-specific IFN-γ and IL-4 cytokines was more sensitive than LPA (82% vs 26% in acute DHR, 65% vs 26% post-recovery). The results of LPA and IFN-γ assays correlated well ($r=0.7$, $P<0.0001$). In contrast to LPA, drug ELISpots showed positive responses despite concurrent immunosuppressive medication in the index case and also when an immunomodulatory culprit drug was tested. In summary, these results demonstrate that the use of assays for drug-specific IFN-γ and IL-4 release in combination with the LPA can aid the diagnosis of drug hypersensitivity during the acute, as well as the post-recovery, phases of the reaction.

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Enzymatic change of autoantibody glycosylation modulates FcγR expression and reverts pathogenic effects of autoantibodies already bound to their skin target

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Glycosylation of the Fc fraction of IgG plays an important role in the interaction with Fcγ receptors (FcγRs). EndoS, derived from Streptococcus pyogenes, exclusively hydrolyzes the glycan of IgG. EnodS has previously been shown to reduce the binding of treated IgG to activating FcγRs whereas the affinity to the inhibiting FcγRIIB was increased. However, the effect of EndoS on autoantibodies that have already bound to their target antigens has not been shown yet. Here, we used different experimental models of the subepidermal blistering autoimmune disease epidermolysis bullosa acquisita characterized by autoantibodies against type VII collagen. EndoS significantly reduced lesions in BALB/c mice when EndoS was co-injected with the pathogenic IgG. Importantly, EndoS suppressed disease activity in the immunization-induced mouse model, closely mimicking the patients' situation, even when anti-collagen type VII antibodies had bound to the skin and lesions had already developed. Elution of skin-bound anti-type VII collagen IgG after EndoS treatment revealed hydrolysis of the glycan moiety. Furthermore, mRNA levels of the activating FcγRs of CD11c-expressing cells in skin lesions were significantly decreased, while mRNA levels of the inhibitory FcγRIIB were increased. Here, we described two novel effects of EndoS: (i) enzymatic glycan hydrolysis may be effective in already tissue-bound pathogenic IgG and (ii) differentially modulates the expression of FcγRs leading to an anti-inflammatory milieu and, subsequently, to reduced tissue destruction. Enzymatic autoantibody glycan hydrolysis warrants exploration as a novel therapeutic option in patients with autoantibody-mediated diseases.

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PC111: a monoclonal anti-Fas Ligand antibody for the treatment of pemphigus

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Pemphigus is a chronic autoimmune bullous disease of the skin and mucous membranes. It is an orphan condition, as the only treatment consists of long-lasting use of systemic immunosuppressors, that associate with severe side effects, potentially leading to patients' death. Apoptosis is definitely involved in the pathomechanisms of pemphigus. Pemphigus autoantibodies (PVIgG) induce the up-regulation of Fas ligand (FasL) mRNA in keratinocytes, and elevated levels of FasL are present in pemphigus sera. We have shown that FasL cleaves desmoglein (dsg)-1 and-3 in keratinocytes, thus causing acantholysis in vitro. By contrast, FasL siRNA treated keratinocytes are protected from PVIgG-induced apoptosis and acantholysis. The aim of this study was to validate the use of the novel anti-FasL antibody (Ab) PC111 in vivo as a new therapeutic tool for pemphigus. Using the "passive transfer" pemphigus mouse model, we showed that PVIgG induced the increase of the FasL active form, starting at 1hr after injection, with a peak at 3hrs in mouse keratinocytes. Mice were then injected with PVIgG alone or in combination with anti-FasL Ab at different times. When anti-FasL Ab was administered simultaneously or 2hrs before PVIgG, blisters continued to form. When anti-FasL Ab was given 1hr after PVIgG, blisters were still visible, but smaller. When anti-FasL Ab was administered 3hrs after PVIgG, when FasL levels are highest, no blister formation occurred. The effect was confirmed by measuring the length of the histologic clefts. By using decreasing doses of anti-FasL Ab 3hrs after PVIgG injection, we set the minimum effective dose at 10μg/mouse of anti-FasL, as the dose that completely inhibits blister formation. Finally, we screened several anti-FasL-producing hybridomas and found the human PTA-4018 as the most effective in cleaving dsgs. The human anti-FasL Ab PC111 is now ready to enter the pre-clinical phases, and it will likely represent a novel tool to treat pemphigus by targeting a pathway downstream the PVIgG-antigen binding.

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Cw6 but not allele LCE3C_LCE3B deletion confers sensitivity to ustekinumab treatment in psoriasis

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Genetic and environmental factors are involved in determining the appearance of psoriasis, many studies have shown the high prevalence of the HLA-Cw6 allele in patients affected by moderate to severe psoriasis suggesting a role for this allele in the immune system dysfunction associated with psoriasis. Recently, the deletion of LCE3B and LCE3C genes (LCE3C_LCE3B-del), members of the late cornified envelope (LCE) gene cluster, was significantly associated with risk of psoriasis, suggesting that compromised skin barrier function may have a role in psoriasis susceptibility. Objective of the present study was to determine whether the presence of HLA-Cw6 allele or LCE3B_3C gene deletion might influence response to the IL-12/23 blocking antibody ustekinumab. To this aim, we have performed HLA-Cw6 haplotyping and assessed the presence of LCE3B and LCE3C deletion in 40 patients affected by moderate to severe plaque psoriasis and treated with ustekinumab for at least 52 weeks. We have evaluated if the response to ustekinumab was associated to the presence of the HLA-Cw0602 allele. Our data indicate a faster initial clinical response to ustekinumab in Cw6 positive patients. At week 4 PASI 50 was reached by 80% of Cw6 positive patients and in 50% of Cw6 negative patients ($p<0,01$), while no difference was observed at week 12 and 24. When considering the presence of LCE3B_C deletion, we haven't observed any significant variation in response to ustekinumab. Similar results were obtained when considering PASI75. Also we have studied the a mutual influence between the presence or absence of the HLA-Cw6 allele and the presence or absence of homozygous or heterozygous deletion of LCE3B and LCE3C finding no significant association. Our results suggest that the immune system-related molecule Cw6 rather than the skin barrier-related molecules LCE3B_C may influence response to the T-cell targeting agent ustekinumab.

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Therapeutic Potential of Oral L-Histidine in Atopic Dermatitis

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The aetiology of atopic dermatitis (AD) has been linked to deficiencies in the epidermal barrier protein, filaggrin (FLG). In mammalian skin, L-histidine (HIS) is rapidly incorporated into FLG ('HIS-rich protein') which aggregates keratin filaments in granular layer cells, 'collapsing' them to form squames that are critical to skin barrier function. Subsequent FLG proteolysis releases HIS as an important Natural Moisturising Factor (NMF). If fed a HIS-restricted diet, healthy subjects develop an eczematous rash. We therefore hypothesised that oral HIS would augment both FLG processing and NMF-mediated skin hydration, enhance skin barrier function and reduce AD severity. Adult AD patients (European AD Diagnostic Criteria; n=24; 58% female; 19-34 yrs) with mild to severe AD were recruited into a randomised, double-blind, placebo controlled, proof of concept (PoC) study. After a 2 wk 'wash-out' (WO) period, subjects were supplied with either oral HIS or placebo (erythritol) to be taken once-a-day with clinic visits after 4 and 8 wks. Oral HIS led to significant improvements in AD disease severity as measured by the validated SCORing AD - SCORAD (34% and 32% reduction from post-WO mean of 31.9; $p<0.003$) and Patient Oriented Eczema Measure (POEM; 38% and 38% reduction from post-WO mean of 18.4; $p<0.002$) tools after 4 and 8 wks treatment respectively. Trans-Epidermal Water Loss (TEWL) a measure of skin barrier function, was also reduced by 19% and 17% after 4 and 8wks of HIS treatment although this did not reach significance. There were no adverse events associated with oral HIST administration. Both the clinician (SCORAD) and patient (POEM) scores in the PoC study indicate that oral HIS has significant therapeutic potential in mild-severe, adult AD. The efficacy of oral HIS is similar to certain mid-potency topical steroids and combined with its safety profile, suggests that it may be a safe, convenient, non-steroidal intervention suitable for potential chronic/prophylactic use in the management of AD in all age groups.

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Anti-IL-23p19 (MK-3222): effects on the hallmarks of inflammation in psoriasis

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IL-23 is a key driver of inflammation in psoriasis released by keratinocytes, DC and macrophages. The present study assessed the safety, tolerability, pharmacokinetics and clinical efficacy of the anti-IL-23p19 antibody MK-3222. Patients either received placebo or MK-3222 at doses of 3 or 10 mg/kg, respectively, at week 0, 4 and 8. To analyze the impact of MK-3222 on the cellular components of the psoriatic skin, we subjected biopsies from a subset of patients (n=22) obtained before dosing and at week 12 to detailed immunohistochemical analysis. Clinically, patients receiving MK-3222 showed dose-related improvement of disease severity, as compared to placebo treatment, up to 100 percent. Psoriatic lesions, when compared to non-lesional skin, were marked by increased epidermal thickness, strong expression of Keratin 16 and Ki-67 in keratinocytes and increased CD31+ vessel density. The dermal inflammatory infiltrate in psoriatic lesions predominantly consisted of CD3+, CD4+, CD8+T cells and CD68+ leukocytes and, to a lesser extent, of BDCA-2+ pDC, CD11c+ mDC and CD15+ neutrophils. After treatment with MK-3222 the observed epidermal alterations in lesional skin resolved and were comparable to non-lesional skin. Remarkably, a significant reduction of CD3+ T-cells, pDC, mDC, neutrophils and macrophages in the inflammatory infiltrate was seen. However, the number of CD8+ T-cells as well as CD207+ LC was not affected. Importantly, psoriatic skin contained a significant amount of p19+ target cells, which were completely abolished after treatment with MK-3222. Our data shows that administration of MK-3222 in psoriatic patients induces a marked reduction of cutaneous inflammation.The clinical and immunohistological improvement observed strongly suggests that targeting of IL-23 by anti-p19 treatment controls downstream inflammatory pathways important for disease development.

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The Effect of Systemic Therapies on Langerhans' Cell Migration in Psoriasis

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Epidermal Langerhans cells (LCs) act as sentinels of the immune system: they migrate from skin to local lymph nodes to present antigen to T cells. In the steady state LCs promote immune tolerance, whereas following challenge they initiate the adaptive immune response. We have shown previously that LC migration in vivo is impaired in the uninvolved skin of patients with early-onset psoriasis. Using a novel epidermal explant model, we investigated the effect of systemic therapies on LC migration in uninvolved skin of psoriasis patients (onset <40y of age). We compared patients who had responded well to either T-cell targeted therapies (ciclosporin, methotrexate); anti-cytokine biologics (adalimumab, etanercept, ustekinumab), or fumaderm, with untreated patients and with healthy controls. Epidermal sheets prepared from biopsies of sun-protected skin were cultured for 24 hours; stained with CD1a and LC counts performed. Langerhans cells migrated from explants of all healthy controls (mean 19.6±3.1%); however in untreated patients there was little or no migration (mean 0.9±1.8%). Migration was impaired in patients who had responded to T-cell targeted therapies (mean -2±4.4%). In contrast there was significant restoration of migration in patients who had responded to anti-cytokine biologics and fumaderm (mean 17.7±3.9%). We hypothesized that changes in availability of local factors within the epidermal environment may be responsible for abrogation of LC migration in untreated psoriasis. We cultured epidermal explants from patients using supernatants derived from the human keratinocyte cell line HaCaT. There was a significant restoration of LC migration in explants cultured in conditioned medium (mean 23.0 ± 4.7%). These data indicate that therapies which target key cytokines involved in the pathogenesis of psoriasis can restore LC migration whereas T-cell targeted approaches do not. It is likely that local, keratinocyte-derived factors may be responsible for the abrogation of LC migration in psoriasis.

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Fluorescence-navigated sentinel node mapping using indocyanine green: report of 50 cases

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Background and objective: Although sentinel lymph node (SLN) biopsy combined use of radioisotope (RI) and blue dye (BD) achieved a high detection rate, approximately 5% of melanomas with negative SLNs develop nodal metastasis (false-negative SLN biopsy). We tested a new lymphatic navigation method using indocyanine green fluorescence imaging (ICG) to detect such "occult" SLNs. Methods: Fifty skin cancer patients received SLN biopsy with the following three methods: RI (99Tc-tin colloid), BD (2% patent blue), and ICG (0.5% indocyanine green). All the tracers intradermally injected at the same location. Lymph nodes detected by any of the three methods were counted as SLNs. Results: ICG detected more SLNs in 14 out of the 50 cases (28%). The average numbers of SLNs detected by ICG, RI, and BD were 2.17, 1.68, and 1.75, respectively. Interestingly, ICG detected more SLNs in one basin, it also detected additional SLNs in other basins not found by the other tracers. Conclusion: ICG detected SLNs more efficiently than the conventional methods, and these "occult" SLNs may offer an explanation for some false-negative cases. We recommend using ICG in addition to a conventional method to reduce the risk of overlooking these "occult" SLNs.

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Analysis of tumor escape mechanisms in melanoma tissues

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We recently reported that tumor escape mechanisms inversely correlated with the functional activation of melanoma-reactive T cell responses. Therefore, insight in these mechanisms is relevant for the potential outcome of immunotherapy. We retrospectively investigated metastatic melanoma tissues of patients before and after receiving autologous GM-CSF-producing tumor cells vaccines, for the expression of tumor escape mechanisms in relation to the clinical outcome. Tumor tissues of 62 metastatic melanoma patients were analyzed by immunohistochemistry for the presence of infiltrating (suppressive) cells (e.g.T cells, mast cells, regulatory T cells), T cell inhibitory factors (galectins, PD-1/PDL-1), cytotoxic molecule granzyme B and tolerogenic cytokines (TNFa, IL-10). Data were analyzed by scoring the percentage of tumor cells or infiltrating cells expressing the markers. Results were related to (progression-free) survival (PFS and OS). Higher numbers of infiltrating T cells were found in the tumor of melanoma patients with prolonged PFS (n=6; median PFS> 6 months; median OS=43 months) compared to patients without PFS (n=10; median OS=15,4 months). Moreover, more granzyme B+ CTLs were present in the tumor of the patients with prolonged PFS, coinciding with prominent active caspase-3 expression in the tumor area, suggesting apoptosis of melanoma cells induced by CTLs. Interestingly, patients without prolonged PFS had significantly higher expression of IL-10 and TNFa in the tumor cells, indicating a tumor environment suppressive for immune cells. Our data show that advanced melanoma patients with prolonged PFS had increased numbers of intratumoral CTLs, corresponding with apoptosis of melanoma cells. Together with low expression of IL-10 and TNFa in the tumor, this might contribute to the anti-melanoma immune response in these patients, resulting in prolonged PFS. Currently, these results are related to the functional T cell responses.

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CD203c expression-based basophil activation test is useful in the diagnosis of wheat-dependent exercise-induced anaphylaxis

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Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a special form of wheat allergy induced by the combination of wheat ingestion and physical exercise. Wheat ω-5 gliadin is a major allergen in conventional type of WDEIA and detection of serum IgE specific to recombinant ω-5 gliadin is a reliable method for its diagnosis. Recently, increased incidence of a new WDEIA subtype caused by hydrolyzed wheat protein (HWP) has been observed. We have encountered several WDEIA patients who were sensitized to HWP primarily through percutaneous and/or rhinoconjunctival routes by using HWP-containing facial soaps. A new subtype of WDEIA has little serum specific IgE to ω-5 gliadin. To establish a predictive in vitro test for differentiating these 2 subtypes of WDEIA, we measured basophil CD203c expression induced by different types of wheat proteins and evaluated the diagnostic efficiency of the reactions in the patients. We examined ten patients consisting of five patients of new subtype and five patients of conventional type. Sensitization to wheat proteins was confirmed by skin prick testing, detection of serum specific IgE, challenge testing, and IgE-immunoblotting. Expression of CD203c on basophils was analyzed by flow cytometry using ω-5 gliadin and HWP. HWP induced CD203c expression on basophils in all the patients of new subtype, whereas no significant CD203c expression in the patients of conventional type. ω-5 gliadin induced CD203c expression in the patients of conventional type, but not in the patients of new subtype. Measurement of CD203c expression on basophils is useful for evaluating differential sensitization to wheat proteins in vitro according to the clinical condition in WDEIA. The basophil activation test based on the expression of CD203c may help determine causative allergens for a wide variety of food allergies.

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Antimicrobial Properties Of Distinctin In An Experimental Model Of MRSA-Infected Wounds

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The emergence of antibiotic-resistant Staphylococcus aureus strains is highly hindering the efficacy of systematically administrated conventional antibiotics. Distinctin is a heterodimeric peptide from the Amazonan frog Phyllomedusa distinct. Aim of the study was to evaluate the efficacy of distinctin in the management of cutaneous methicillin-resistant Staphylococcus aureus wound infections in an experimental mouse model. Wound, established through the panniculus carnosus of BALB/c mice, was inoculated with colony-forming units of methicillin resistant Staphylococcus aureus. Mice were treated with topical distinctin (1 mg/kg of body weight), topical teicoplanin (7 mg/kg of body weight), intraperitoneal teicoplanin (7 mg/kg of body weight); topical teicoplanin and daily intraperitoneal teicoplanin; topical distinctin and daily intraperitoneal teicoplanin. Bacterial cultures of excised tissues and histological examination of micro-vessel density and of VEGF expression were performed. The combination between topical distinctin and parenteral teicoplanin showed to inhibit bacterial growth to levels comparable with those observed in uninfected animals. Wounded areas of animals treated with distinctin were characterized by a more mature granulation tissue with more organized and a denser type of connective tissue, compared to mice treated only with teicoplanin . Treatment with topical distinctin resulted in a significant impact on VEGF expression and micro-vessel density. In conlusion the combined use of distinctin with teicoplanin, may be useful in the management of infected wounds through a significant bacterial growth inhibition and an accelerated repair process.

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Loss of epidermal Langerhans cells caused by niacin deficiency: the pathogenesis of pellagra as another member of trophic skin disorders

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Deficiency dermatoses include acrodermatitis enteropathica, necrolytic migratory erythema and pellagra. Acrodermatitis enteropathica is caused by Zinc deficiency, necrolytic migratory erythema is most commonly caused by glucagonoma. Pellagra is caused by niacin deficiency and characterized by photosensitive dermatitis, diarrhea, dementia, however, the exact pathomechanism of these symptoms is not yet known. From the histological resemblance of epidermal lesions of these trophic diseases, we suspected the involvements of Langerhans cells in pellagra, whose loss is also noted in acrodermatitis enteropathica recently. We examined the presence of various cells in the skin lesion of patients with pellagra, and confirmed the disappearance of dendritic cells componens by immunohistochemical staining for langerin, HLA-DR, CD1a and s-100. We show the degeneration of kerationcytes in the upper spinous layer due to apoptosis by TUNEL method and cleaved caspase 3. We then analyzed the temporal change of the dendritic cells in the skin and pellagra model mice, which are treated with 6-aminonictininamide. In these pellagra model mice, epidermal and dermal dendritic cells were almost completely lost after treatments. Pellagra model mice and normal mice was treatment with 10% Benzalkonium chloride or 0.5%Dibutyl squarate on the both sides ears. Swelling responses were quantified (ear thickness minus pretreatment ear thickness) by using micrometer. Pellagra model mice showed the modestly decreased allergic response to 0.5% Dibutyl squarate compared with normal mice, while the irritant response to 10% Benzalkonium chloride was markedly prolonged in pellagra mice. Furthermore, the pellagra mice showed delayed sunburn after the irradiation of 1J/cm2 UVB compared with normal mice. From the observations, we supposed that losses of dendritic cells are the common and primary pathophysiological mechanism of the trophic skin diseased including pellagra.

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Induction of Th1 and Th17 cytokines in cultures of circulating CLA+ T cells and epidermal psoriatic cells activated by streptococcus depends on MHC class II and MHC class I

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Streptococcus pyogenes infection is associated with the onset of psoriasis; however the mechanisms generating Th17 response and keratinocyte activation/proliferation under such circumstances are poorly characterized due to the lack or relevant models. We have previously shown that ex vivo activation of circulating CLA+ T cells and epidermal cells derived from autologous lesional skin with streptococcus induces Th1/Th17 cytokine production together with epidermal cell activation and hyperplasia, in contrast to CLA- from same patients and memory T cells from controls. Among many other different mediators generated during this activation, we have analyzed by multiplex fluorescence bead-based immunoassay IL-6, TNF-alpha, IL-17A, IL-17F and IFN-gamma in 5 days supernatants from psoriatic patients. Under such activating conditions, novel basal keratinocytes expressing CD29 and HLA-DR were generated analyzed by CFSE. The cytokines IL-17A, IL-7F, IFN-gamma, IL-6, and TNF-alpha reached net values of 33 ± 13, 435 ± 242, 72 ± 49, 264 ± 295 and 4324 ± 1847 (pg/ml), respectively. The blockade of MHC class II or class I with specific antibodies reduced the generation of novel basal keratinocytes and cytokine production by 90% and 50%, respectively. Lymphocytes and keratinocytes from guttate psoriasis patients with onset associated with pharyngeal streptococcus infection, ASO levels>200 and HLA-Cw6 tended to have a higher stimulation ex vivo. Interestingly, cytokine production correlated with ASO levels r=0.7-0.9. Since this model gathers some of the elements involved in the early plaque formation, we consider that it could be useful to characterize and to identify initial mechanisms taking place during psoriatic plaque formation.

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Downregulation of the Intracrine Skin Glucocorticoid Pathway in Psoriasis

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Glucocorticoids (GC) induce powerful immune-modulatory effects and are used to treat inflammatory skin diseases including psoriasis. We previously demonstrated de novo cortisol synthesis in normal primary human keratinocytes (NK). The purpose of this study was to examine whether the local GC pathway is aberrant in psoriasis. Here we show that the GC pathway is downregulated in human psoriatic skin. Expression of steroidogenic acute regulatory protein was decreased in both lesional and uninvolved psoriatic skin. Thin layer chromatography showed reduced pregnenolone to cortisol metabolism in primary psoriatic keratinocytes (PK) over 24h (38.8±6.1% NK vs. 8.7±0.4% uninvolved and 8.6±0.2% lesion PK, P≤0.01). In addition, steroid sulphate levels in PK increased (10.5±1.7% NK vs. 19.5±1.9% uninvolved and 22.8±3.0% lesion PK, P≤0.05), suggesting greater steroid clearance from the system. LC-MS/MS analysis of cortisol release from whole skin mounts over 24h was less than 10% in psoriatic samples relative to normal skin; normal skin 809.6±120.4ng/ml, psoriatic lesion 67.7±11.8ng/ml, psoriatic uninvolved 63.1±7.9ng/ml. Western analysis of steroid enzymes was unable to detect 3bHSD1 expression in uninvolved and lesion psoriatic epidermis but was expressed in normal skin. In addition immunohistochemistry revealed glucocorticoid receptor (GR) expression was down regulated in lesion psoriatic skin but not uninvolved or normal skin. We can now conclude that the local GC pathway from synthesis to GR expression is attenuated in psoriatic skin. Decreased GC synthesis and response in psoriatic skin would provide an environment favourable for the promotion of inflammation that is well defined in psoriasis. Therefore dysregulation of localised skin GC pathway may be implicated in the pathogenesis of psoriasis. Better understanding of the local GC pathway represents a novel therapeutic target and could limit the side effects that are associated with GC therapy.

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Alterations in dendritic cells subsets of psoriatic patients before and after biologic therapy

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Dendritic cells (Dcs) are focus of interest in psoriasis research. Previous studies showed that in the inflamed dermis of psoriatic patients, there is an increase in DCs probably derived from circulating precursors. To further investigate their role in pathogenesis of disease we analyzed peripheral DCs, specifically myeloid DCs (mDCs) and plasmocytoid DCs (pDCs), in psoriatic patients treated with anti-TNF-alpha therapy. Circulating mDCs and pDCs levels were evaluated by flow-cytometry in 8 patients (4 males and 4 females, age range 44-80) affected by moderate to severe psoriasis (PASI score >10) before and after 6 months of infliximab therapy. In addition, 4 healthy blood (matched with patients for sex and age) were included in the study. The mDCs and pDCs were identified by the concurrent HLA-DR expression and mutually exclusive membrane expression of BDCA-1 or BDCA-2, respectively. The expression of CD86 was also analyzed. Untreated psoriatic patients are characterized by a quantitative deficit in their peripheral circulating mDCs (3.2 ± 0.3% of total PB mononuclear in untreated psoriatic patients versus 6.8 ± 4.5% in the healthy control group - p<0.05) and pDCs (1.3 ± 0.4% versus 2.3 ± 41.1%). Responders psoriatic patients (after 6 months of therapy) showed a significant increase in their numbers of circulating mDCs (from 3.2% ± 0.3% to 7.8% ± 1.8% - p<0.05) and pDCs (from 1.3% ± 0.4% to 3.8% ± 2.7%). Our preliminary results, although in a limited number of patients, suggest that in psoriasis peripheral blood was characterized by a decreased number of both mDC and pDC subsets and an increased number after biological therapy. According to our study this alteration in circulating DCs could correlate with the ongoing of the disease and the response to biological therapy.

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Extracorporeal photopheresis in chronic graft-versus-host disease

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Chronic graft-versus-host disease (cGvHD) remains a major complication of allogeneic hematopoietic cell transplantation involving multiple sites and occurring in approximately 50% of transplant recipients, with a significant negative impact on quality of life and patient survival. Extracoporeal photochemotherapy (ECP) has been shown to ameliorate the signs and symptoms of patients with cGvHD. The aim of the study was to test it on a subset of patients with moderate or severe cGvHD, recalcitrant to traditional therapies, if ECP could have a role in long term control of the disease. 69 patients with moderate or severe recalcitrant cGvHD were treated with ECP for at least 2 years. Cutaneous, oral and global disease activity was scored before each ECP cycle by means of National Institutes of Health Criteria for Clinical Trials in cGvHD. Quality of life before treatment with ECP, after 6, 12 and 24 months was scored by means Karnofsky Performance Scale Index (KPS). Our data showed that ECP is an effective therapy in the treatment of cutaneous and oral cGvHD. Moreover, ECP allows to obtain a statistically significant improvement of the global score and therefore of the quality of life. This latter finding is also demonstrated by statistically significant changed of the KPS at 12 months. Our data also confirm that ECP may a have steroid-sparing effect in the treatment of chronic GVHD. Only 7 patient were withdrawn from the study for unresponsiveness. No clinical signs of immunosuppression or other severe adverse events became evident. Our study confirms the low risk of side effects and the long lasting anti inflammatory, immunomodulating effects of ECP in patients with moderate or severe cGvHD. However multicenter controlled trials with a standardized protocol are lacking which leads us to believe this treatment should be reserved for patients with moderate or severe cGvHD, recalcitrant to traditional therapies.

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Localized scleroderma and ultraviolet A1 therapy: immunohistochemical analysis and role of decorin

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The objective of our study was to evaluate the effects of ultraviolet A1(UVA1) irradiation (340-400 nm) on decorin, a leucine rich proteoglycan (SLRP) with antifibrotic activity, in a group of patients affected by localized scleroderma (LS). In particular, we considered the immunohistochemical expression of decorin before and after UVA1 therapy in order to evaluate if photo-induced modulation of decorin exerts a key role in the progression of LS. Ten female patients with LS were treated with medium-dose (50J/cm2) UVA1. In total, 30 treatments and 1500J/cm2 cumulative UVA1 doses were performed in all patients. Clinical evaluation was performed using the modified Rodnan skin score (MRSS). Skin thickness was also determined by 10MHz ultrasound examinaton. Moreover skin elasticity, resilience, hysteresis and tensile distensibility were evaluated at baseline and after the treatment with an elastometric valutation using Dermalab USB (Cortex Technology). All the measured parameters showed an improvement after the therapy. Histological and immunohistochemical analysis of skin were performed in all the patient at baseline and on completion of the course of UVA1 therapy. Immunohistochemical analysis was performed using a DSPG2 clone of decorin (Sigma Aldright). Histological examination performed before and after the treatment showed a significant reduction of the monocyte infiltrate and of vascular lesions with a substantial reduction of fibrosis, especially in the reticular dermis where the collagen fiber bundles were more spaced. The immunohistochemical staining for decorin showed, after the treatment, an increased amount of the proteoglycan, with a mild positivity in the papillary dermis, and a strong positivity in the reticular dermis with collagen fibers very similar in appearance to normal skin. Our results showed that UVA1 therapy has a positive effect in the fibrillogenesis of collagen and that decorin has a key role within the same process.

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Interleukin-8 content in the stratum corneum is an good indicator of the severity of inflammation in the lesions of atopic dermatitis

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Atopic dermatitis (AD) is an inflammatory skin disease, characterized by both acute and chronic eczema. Various markers are used to clinically evaluate the severity of AD. In order to identify a marker of local severity of AD, we measured IL-8, IL-18, vascular endothelial growth factor (VEGF), and transforming growth factor-α (TGF-α) levels in the stratum corneum (scIL-8, scIL-18, scVEGF, and scTGF-α) and evaluated the correlation between the levels of these cytokines and the clinical severity scores of localized skin lesions. Stratum corneum samples were collected from the skin lesions of 50 patients with AD using the tape-stripping technique, and the scIL-8, scIL-18, scVEGF, and scTGF-α levels were evaluated using the ELISA method. The levels of scIL-8, scIL-18, scVEGF, and scTGF-α were significantly higher in patients with AD than in healthy controls. Additionally, the levels of scIL-8, scIL-18, and scVEGF significantly correlated with the severity of AD. Among these cytokines, scIL-8 showed the highest correlation with the severity scores of lesions in AD as well as other parameters. Our results suggest that measuring cytokines in the stratum corneum by using ELISA combined with tape stripping is a convenient method to evaluate the severity of skin lesions in AD.

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Immunohistochemical Localization of Glucocorticoid Receptor Alpha and Beta in Inflammatory Dermatoses

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Immunohistochemical localization of GRalpha and GRbeta has not previously been studied in inflammatory dermatoses. Alternative splicing of glucocorticoid receptor (GR) pre-mRNA generates GRalpha, but also GRbeta, which does not bind glucocorticoids but antagonises the activity of GRalpha in a concentration depended way. Ligand-free GRalpha receptor is located in the cytoplasm as a multi-protein complex, where it binds the hormones, translocates to the nucleus and through binding to specific glucocorticoid response element (GRE) modulates the expression of glucocorticoid-responsive genes. GRbeta can localize both in the cytoplasm and nucleus. GRbeta mRNA has been found in a variety of human cells, but GRbeta protein has been shown to have a more restricted cellular distribution, frequently it has been found in healthy T lymphocytes, macrophages, neutrophils, eosinophils and endogenous peripheral mononuclear cells. 62 cases of inflammatory dermatoses were selected from the files of pathological specimens (Department of Pathology of Oulu University Hospital). All of the samples were stained with GRalpha and 60 of the samples with GRbeta. Three cases of normal skin specimens were used as control samples. Formalin-fixed, paraffin-embedded, 3-4 um thick sections were mounted on precoated slides and immunohistochemically stained using Dako Real EnVision IHC -method. Commercially available antibody was used for staining with GRalpha and GRbeta-specific polyclonal rabbit antibody raised against human GRbeta was used for staining with GRbeta. As expected, staining for GRalpha was mostly cytoplasmic and the signal was found in all cell types studied. As previously described, GRbeta protein can be localized in both nucleus and cytoplasm. In these specimens staining was mostly cytoplasmic, but especially neutrophil nuclei also stained widely positive. Our study describes for the first time localization of GRalpha and GRbeta in inflammatory dermatoses.

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Epigallocatechin-3-gallate suppresses insulin-like growth factor-I-induced lipogenesis and cytokine expression in SZ95 sebocytes

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Acne vulgaris is the most common disease of the pilosebaceous unit. The pathogenesis of this inflammatory disease is complex, involving increased sebum production and perifollicular inflammation. To identify effective agents for factors that induce acne vulgaris, we explored the pharmacological potential of epigallocatechin-3-gallate (EGCG), which has been widely investigated as an anti-proliferative and anti-inflammatory agent. In this study, we demonstrated that topical application of EGCG to rabbit auricles reduced the size of the sebaceous glands. When applied to cultured human SZ95 sebocytes, EGCG strongly suppressed cell proliferation and lipogenesis. These actions of EGCG were reproduced in insulin-like growth factor (IGF)-I-differentiated SZ95 sebocytes. To investigate the anti-inflammatory potential of EGCG, we evaluated pro-inflammatory cytokine synthesis in IGF-I-differentiated SZ95 sebocytes and found that expression of IL-1, IL-6, and IL-8 was decreased. These results provide early evidence that EGCG is an effective candidate for acne therapy whose mechanisms of action in IGF-I-differentiated SZ95 sebocytes include the inhibition of lipogenesis and inflammation.

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Adipose Tissue-Derived Mesenchymal Cells for the reparation of major facial traumatic deformities

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Purpose of the present work is to report the use of autologous adipose-derived mesenchymal cells (ADMC) for the reparation of major posttraumatic facial defects. In recent years, a number of cells resident in adult tissues have been characterized, isolated and used for tissue regeneration. Mesenchymal cells (MC) have a multitude of physiological roles: to differentiate or transdifferentiate into multiple cell types, to modulate immune functions, and to participate in various effects. They can migrate into injured or inflamed tissues, where they down-regulate the availability of proinflammatory cytokines and promote the survival of affected cells, while they produce a variety of growth factors. Bone marrow is the classical tissue for the isolation of MC. Recently adipose tissue was recognized as an alternative source, providing a larger number of cells, being more easily accessible, while limited liposuction is a less traumatic and painful procedure than bone marrow aspiration. In the present study, we report the use of ADMC for the reconstitution of a patient with massive post-traumatic hemifacial defect. A 42 years old man, after a major traumatic injury, was presented with a loss of volume of the face, very thin skin and no mobility of the facial nerve, after the initial bone and soft tissue reconstruction. In the impossibility to perform secondary reconstruction procedures, we have applied ADMC, firstly a suspension of ADMC and isolated adipocytes and two months later pure homologous ADMC. Clinical and laboratory assessment showed that there was a volume restoration of the face, an amelioration of the thickness of the skin and, the major finding of our study, an improvement of the mobility of the injured facial nerve. We propose local application of homologous ADMC as a valuable alternative to other reconstructive methods of treatment.

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CD8+Tumor-infiltrating lymphocytes at primary site were the important prognostic factors of cutaneous angiosarcoma

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To investigate our hypothesis that the subsets of tumor-infiltrating lymphocytes (TILs) have a relation with the clinical stages or the prognosis of patients with angiosarcoma, immunohistochemical analysis of TILs at all stages of cutaneous angiosarcoma was performed. Non-treated primary cutaneous anigosarcoma specimens from 36 patients at stage 1 with no metastases, 4 patients at stage 2 with lymph node metastases and 11 patients at stage 3 with distant metastases were evaluated retrospectively by immunohistochemistry stained CD4, CD8, and FOXP3. Because the prognosis and the numbers of TILs were different in each stage group, we evaluated TILs at each stage. Stage 1 patients with higher numbers of CD8+ TILs in their primary tumors demonstrated longer survival compared with patients with lower ones, estimated by the Kaplan-Meier method and the log-rank test based on the number of subsets of TILs. The higher numbers of CD8+ TILs had positive correlation with the longer lung metastasis-free period. Moreover, the higher numbers of CD8+ TILs in stage 3 patients had still positive correlation with their survival months. To compare the frequency of CD8+ effector T cells with healthy controls and melanoma patients as a disease control, peripheral blood mononuclear cells (PBMC) were assessed by flow cytometry. Interestingly, the percentages of CD8+ T cells producing IFN-γ in PBMC were significantly higher in angiosarcoma patients compared with those of not only healthy controls but also melanoma patients. This study provides the importance of CD8+ TILs at primary cutaneous lesions at each stage in the prognosis of patients with angiosarcoma.

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Significance of ultrasound morphologic evaluation of basocellular cancers from the viewpoint of the therapeutic modalities and followup procedures

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Introduction High frequency probes fine flow technique and tissue elastography are equally compulsory elements int he diagnostic workup of cutan lesions. In the therapeutic planning process of the most frequent cutan tumour namely the basaliomas the ultrasound morphology plays significant role. Ultrasound characteristics which, beside its cardinal differential diagnostic role, also plays an important role int he classification process of basaliomas. The ultrasonographic structure of basaliomas shows typical specific picture that helps differenciate various histologic types during thetherapeutic decision making. Patients and methods: 367 patients with basalioma were evaluated with 18 MHz frequency linear transducer between 01 2010 and 05 2012. Depth of invasion, dermal contour, vascularity and tissue elasticity of lesions were evaluated. Sonographical findings were compared to histopathological findings. Above these criteria localisation, type, focality of the lesion and patient age were analized, and based on that, the onko-board decided on the therapeutic modality of choice to be the most effective for the patient. At most cases with superficial lesions having sharp contour surgical excision was chosen, lesions of multifocal type or contour irregularity on ultrasound or showing superficial spread pattern on pathology study were treated by surgery combined with irradiation with providing onologists the correct status of the surgical borders, chemotherapy was also offered to this population of patients.USG showed 97% correlation with histologcal results. Nodular and infiltrative types of basaliomas were correctly predicted by US. Conclusions There was a statistically significant correlation between the histopathologic result and ultrasonographic morphology of the basaliomas in the examined patient population. The therapeutic aproach based upon our method led to a small failure rate during the follow up period.

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Circulating endothelial cell levels in psoriatic patients before and after treatment with Etanercept

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To explain the association between psoriasis and cardiovascular diseases, it has been supposed that systemic inflammation of psoriasis may cause itself insulin resistance, which in turn triggers endothelial cell dysfunction, leading to atherosclerosis and finally to myocardial infarction or stroke. Circulating Endotelial Cells (CECs) are cells shed from injured vessel wall as a consequence of pathological processes causing a damage to the endothelium and their levels have been found to be predictor of major cardiovascular events and death. Considering CECs as a possible marker of endothelial damage/dysfunction, aim of this study was to evaluate their serum levels in psoriatic patients, without any conditions already known to be associated with high CECs levels. CECs levels were also measured before and after treatment with a TNF alpha blocking agent, etanercept, in order to evaluate a possible effect of anti-TNF alpha treatment on endothelial wall. The CellSearch system was used for the immunomagnetic isolation of CECs in the bloodstream. We enrolled 48 patients affected by psoriasis and 18 healthy subjects as controls. A higher count of CECs (in 4 ml) was found in psoriatic patients than in healthy subjects (42,4 ± 37,9 vs 15,5 ± 4,4) (p= 0,009). A significant reduction in CECs levels was observed after six months (13,8 ± 5,3) of therapy in 15 psoriatic patients who were treated with etanercept. These findings further sustain the hypothesis that psoriasis is associated with an endothelial damage/dysfunction that could contribute to a high risk of cardiovascular disease. Considering the reduction in CECs levels that with observed during the treatment with etanercept, we can suppose that anti-TNF alpha agents exert a protective effect on the vessel wall directly by interfering with the atherosclerotic process or indirectly trough a reduction of inflammation of psoriasis.

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Itch characteristics and its association with psoriatic patients' quality of life

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Current data indicate that itch is present in 70-90% psoriatic patients. The aim of the study was to evaluate itch in relation with selected cytokine serum levels (IL-12, IL-23, IL-17a, TNF alfa), disease severity and patients quality of life. 60 psoriatic patients (30 females, 30 males; age range: 20-70 years; mean+/-SD 44.91+/-14.78) and 48 healthy controls (21 females, 27 males; age range 19-50 years; mean+/-SD 31.38+/-9.01) took part in the study. Duration of the disease ranged from 3 months to 54 years; mean+/- SD 18,76+/-13.50. Disease severity was evaluated by PASI Score and ranged from 0.6 to 27.4 (mean+/-SD 9.32+/-5.97). Itch was estimated by Itch Severity Evaluation Questionnaire and ranged from 0 to 18 (mean+/-SD 7.0+/-4.47). There were used laboratory methods (ELISA test) to assess cytokine serum levels and questionnaires to estimate quality of life – generic SF-36 (Short Form-36, Health Survey) and dermatological method SKINDEX-29. All the obtained results were analyzed statistically and the statistical significance was set at $p<0.05$. Comparing cytokine serum levels (IL-12, IL-23, IL-17a, TNF alfa) their concentrations were statistically higher in psoriatic patients than in the control group. There were also statistically significant differences between females and males in QoL worse in women. There was observed the difference in IL–12 level – it was higher in women. Analyses revealed a relation between itch range, itch severity, constant itch, global itch and higher cytokine IL-12 level. Itch correlated negatively with physical, emotional and social functioning. In conclusion our study further points out at the importance of assessment and management, and QoL in psoriasis patients.

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Insights into the function of etanercept in psoriasis: a study on slan (6-sulfo-LacNAc) expressing inflammatory dermal dendritic cells and their presumed precursors in blood

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Dermal dendritic cells (DCs) play a central role in the immunopathology of psoriasis. We previously identified slan (6-sulfo LacNAc) DCs as proinflammatory DCs in human blood and as an inflammatory dermal DC subset in psoriasis. Blockade of TNFalpha by biologic agents is one of the most powerful therapies for psoriasis. However, it is not known whether and how these biologics influence proinflammatory slanDCs. We therefore analysed slanDCs in skin and blood during a 24 week treatment period of 10 patients suffering from psoriasis vulgaris with etanercept. In addition, the effect of etanercept on slanDCs purified from blood of healthy donors was studied. SlanDC numbers being increased in active psoriasis skin lesions were significantly reduced in skin lesions after 4 weeks of treatment. In parallel we observed a reduction in the numbers of TNFalpha-, IL-23p19- and CD11c- expressing cells in psoriatic skin. After 24 weeks of treatment the numbers of slanDCs returned to that of healthy skin. However, the fraction of slanDCs expressing TNFalpha and IL-23p19 remained constant between 60 and 70% and the percentage of slanDCs among all dermal IL-23p19 positive cells even increased suggesting their sustained activation. This was supported by the lack of significant costaining of slanDCs and the apoptosis marker active caspase3. With treatment the percentage of slanDCs in blood increased from 0,9 to 2,8% of PBMC suggesting their reduced recruitment to the skin. HLA-DR expression on slanDCs was reduced during treatment with etanercept. In vitro etanercept efficiently downregulated the capacity of blood derived slanDCs to produce IL-1beta, IL-6, IL-23 and IL-12p70. This study for the first time documents the immunomodulatory potential of etanercept on a distinct and highly pro-inflammatory population of DCs in skin and blood of psoriatic patients.

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Stress evaluation in adult patients with atopic dermatitis using salivaly cortisol

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Atopic dermatitis (AD) is a common inflammatory skin disease with recurring episodes of itching and a chronic relapsing course. The symptoms of AD are often aggravated by stress and AD can also lead to psychological stress, such as social isolation, and discrimination. The evaluation of stress biomarkers is helpful to control skin inflammation by a more proactive management in AD patients. The salivary cortisol level is a psychological stressor and it is a better index of chronic stress. This study, measured salivary samples for cortisol in patients with AD (n=25) and compared them with healthy control subjects (n=42). AD patients were also evaluated for general disease severity using the SCORingAD (SCORAD) score. The serum TARC level, serum total IgE level, serum lactate dehydrogenase (LDH) level, and peripheral blood eosinophil count were measured by laboratory tests. The Skindex-16 was used as a skin disease-specific instrument. The results showed saliva cortisol level was significantly increased in AD patients in comparison to healthy subjects ($p<0.01$). The salivary cortisol level was significantly correlated with SCORAD ($r = 0.42$, $p< 0.05$). TARC and LDH were positively correlated with SCORAD. However, no statistically significant correlations were observed between salivary cortisol level and Skindex-16. Saliva sampling has the advantage of being non-invasive, making multiple sampling easy and stress free. These results suggest that the saliva cortisol level is a useful biomarker to evaluate the stress in AD patients.

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Withdrawn

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Enhanced topical delivery of stem cell derived growth factors by elctroporation, fractional laser and microneedle fractional radiofrequency

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The use of growth factors in skin rejuvenation is emerging as a novel anti-aging treatment. However, it is well documented that hydrophilic molecules larger than 500 Dalton (Da) molecular weight have very low penetration through the stratum corneum. Most growth factors are large hydrophilic molecules greater than 20 kDa molecular weight; thus, penetration through the epidermis is an important matter to apply them for skin rejuvenation. In this study, we conducted electroporation, fractional CO2 laser, and microneedle fractional radiofrequency (RF) to enhance skin penetration of stem cell conditioned medium. To confirm transdermal absorption and compare the effect of each procedure and treatment parameter on the permeation enhancement, proteins in stem cell conditioned medium were labeled with fluorescent dye, and were applied on the female miniature pig skin after each procedure. Histologic changes by stem cell conditioned medium through microchannels, which were produced by fractional laser and RF, were also investigated in photo-damaged skin. As a result, fractional CO2 laser and fractional RF significantly increased the cumulative amounts of proteins in stem cell conditioned medium both in epidermis and dermis. The amounts of protein-fluorescent dye conjugates were mainly affected by the depth of microporation, which was dependent on the fluence of fractional laser and needle depth of fractional RF. Penetration after electroporation was confined to the upper spinous layer of epidermis. Histologic examination after stem cell conditioned medium and fractional RF revealed marked increase in dermal thickness, dermal collagen content, and dermal fibrillin-1 content. In conclusion, fractional laser and RF microporation enhances the topical delivery of growth factors with high molecular weight. Thus, this technology can effectively improve photo-induced wrinkle formation by enhancing topical delivery of active agents.

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Tazarotene cream in lamellar ichthyoses: a randomized, double-blind, vehicle-controlled and dose-finding clinical study

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There is a medical need for an effective and safe therapy of autosomal recessive congenital ichthyoses (ARCI). Thirty (30) patients with ARCI were enrolled in a prospective, randomized, double-blind, comparative and multicentric study. An intra-individual design (left vs right) was performed in which patients were requested to apply either Tazarotene 0.05% or Tazarotene 0.1% cream on one side, and their vehicle on the other side, once a day for 4 weeks. A treatment-free period of 8 weeks followed. At each visit, the severity of the scales, as illustrated by a photograder, and roughness on each side was independently assessed, according to a 4-point scale. All patients had lesions of moderate (score 2) or severe (3) intensity for each parameter at baseline. Treatment response, as defined as a remission of the two parameters (0 to 1) associated with a reduction of the score for scaling of at least 2 points at the end of treatment, was observed in 6 patients under Tazarotene 0.05% (N=15, 40%), 8 patients under Tazarotene 0.1% (N=15, 53%) and 3 patients under vehicle (N=30, 10%). Responses in both active groups were found to be statistically significant compared to the vehicle group ($p < 0.03$), and non significant between themselves. Time-to-relapse occurred between 4 and 8 weeks after treatment in the majority of cases (no statistical significance between groups). Adverse drug reactions were all local (skin irritation, skin erosions) and easily resolved by reducing the frequency of product application. In conclusion, Tazarotene cream was demonstrated to be efficacious and safe in patients with ARCI.

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Beneficial Impact of Effective TNF-Alpha Inhibitor Treatment on the Arterial Intima-Media Thickness in Psoriasis

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Carotid intima-media thickness is a reliable surrogate marker of early atherogenesis. Due to systemic character of inflammation immune mediated inflammatory conditions have been associated with increased susceptibility to enhanced atherosclerosis and manifest cardiovascular diseases. A decreased risk of cardiovascular complications have been observed in rheumatoid arthritis following long-term TNF-alpha inhibitor treatment. There are only few data on the efficacy of TNF-alpha blockers in reducing carotid intima-media thickness in psoriatic arthritis. We aimed to evaluate the role of TNF-alpha inhibitors in influencing arterial wall inflammation in severe psoriasis vulgaris. Carotid and brachial intima-media thickness was measured by high-resolution B-mode ultrasonography in 13 severe psoriatic patients (median age: 40 years, mean initial PASI: 25,56) before therapy and after 6-month-long treatment. Statistical significance of the difference between initial and 6-month values was determined for all the observed arteries collectively as well as separately in the carotid and brachial artery group. Therapy improved skin symptoms in all patients which was clinical marker of antiinflammatory effect of the drugs. (mean 6-month PASI: 1,15; mean 95,5% PASI improvement) A significant difference was detected between intima-media thickness values measured before starting therapy and at the end of 6th month when involving all the followed arteries. (p=0,000150) Similarly, initial and follow-up data differed significantly at individual analysis of carotid (p=0,011032) and brachial (p=0,005652) arteries. We suggest that effective blockade of TNF-alpha mediated inflammatory cascade may be beneficial in reducing arterial wall inflammation and consequently the progression of atherosclerosis as well as high frequency of cardiovascular events associated with severe psoriasis. Further prospective investigations with more patients and a long-term follow-up period are necessary.

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Studies on severe adverse drug reactions from allopurinol

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Among us allopurinol is the main cause of severe cutaneous adverse drug reactions (SCADR). To understand their pathophysiology, we performed HLA typing and patch testing in patients, and in vitro studies with THP-1 cells using allopurinol and its main metabolite oxypurinol. We studied 25 patients with SCARDs imputed to allopurinol, 3 Stevens-Johnson/toxic epidermal necrolysis, 18 DRESS and 4 maculopapular exanthema. Patch testing with allopurinol and oxypurinol at several concentrations and vehicles was negative in all patients. HLA typing, performed by PCR-RSSO in 25 patients and 12 controls exposed to allopurinol with no adverse effects, revealed HLA-B*58.01 in 12 patients (48%) and 1 control (8.3%) for a prevalence of 1.96% in the normal population. THP-1 cells were stimulated for 24 h with 0.75 mM allopurinol and 2.5 mM oxypurinol, concentrations that caused a maximum of 30% reduction in cell viability. The mRNA levels of CD40, CD83, CD86, CCR7, IL-12p40, TNF-α, IL-1α, IL-8 and HMOX-1, evaluated by quantitative Real-time RT-PCR, revealed a very significant increase in HMOX-1 and, particularly, IL-8, when comparing non-stimulated to stimulated cells. IL-8 mRNA levels increased by 11 and 30 fold, respectively, for allopurinol and oxypurinol. SCADRs induced by allopurinol are very probably T-cell mediated, in analogy to those induced by other drugs, even though patch testing was negative. HLA B*5801 is a predisposing factor, but with a lower relative risk than HLA B*5701 for abacavir. Allopurinol and oxypurinol directly stimulate THP-1 cells, inducing genes dependent on Nfr2 oxidative pathway, like IL-8 and HMOX-1, which are also activated by contact allergens. As for other drugs and contact allergens, allopurinol may induce oxidative stress in antigen presenting cells, promoting their activation and therefore initiating an adaptive immune response.

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Autologous fat tissue transfer for the reconstruction of face atrophy in Linear Scleroderma

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The purpose of the study is to describe a simple minimal invasive procedure for the repair of the loss of volume of the face and the improvement of the atrophic skin lesions in patients with scleroderma. Scleroderma literally means "hard skin." It is an uncommon disorder of unknown origin of the connective tissue characterized by inflammatory and fibrotic changes in the skin, blood vessels and skeletal muscles. There are two major types of scleroderma -localized and systemic - and each type has many subtypes. The prognosis is generally good. The diagnosis is based in the typical skin changes, the laboratory tests and the skin biopsy. We report the case of a 22-year-old female, who admitted to our clinic with a bilateral band-like skin depression in the zygomato -parietal region of the face due to incipient sclerodermia. Autologous fat transfer was performed. Fat tissue was taken from the patient's lumbar area with a liposuction under local anaesthesia. The fat tissue has been filtered through gauze and then inserted below the dermis of the affected areas of the face. The procedure has been repeated nine months later. The clinical assessment has been made every two months. The results are very good in cosmetic and functional aspects without any complication. Little regarding treatment is mentioned for linear scleroderma in the literature. The conservative and surgical treatments which have been used till now have poor results. Autologous fat grafting is a standard method for soft tissue augmentation and correction of depressed lesions and atrophy. This procedure can be performed on an outpatient basis with local anesthesia. We propose autologous fat tissue transfer for the reconstruction of face atrophy in patients with linear scleroderma, as a simple minimal invasive procedure, with low cost, very good in cosmetic and functional aspect, with minimal donor-site morbidity and without any complications.

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Clinical characterization at onset of childhood psoriasis in Sweden – a population based study

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Age at onset is a disease modifying factor in psoriasis. Genetic as well as clinical differences have been shown in patients with onset in childhood compared with those with adult onset. A peak of onset at puberty has been reported and the genetic background is strong in patients with adolescent onset of disease. In addition to the genetic background, epidemiological factors may impact the outcome of psoriasis and affect the clinical presentation. Epidemiological knowledge surrounding childhood psoriasis is steadily increasing but data captured at onset is still unsatisfactory. Therefore we have established a cohort of children (n=96) recruited within 12 months after onset of disease before the age of 16 years (mean age 8.4 years, range 2 months to 15 years) for prospective follow-up. In this cohort, plaque psoriasis was the most common phenotype (68%) which is in accordance with previous studies of childhood psoriasis. As much as 89% of patients showed lesions in the scalp and 46% had facial lesions. Nails were affected in 11% and joint involvement verified by a rheumatologist was diagnosed in 7% of patients. A first degree relative with psoriasis was reported in 40% of patients and a first or second degree relative in 59% of patients. A history of flexural eczema was reported in 15% and a history of allergy or asthma in 23%. Thus flexural eczema and allergy/asthma was fairly common in our cohort of children with psoriasis. None of the patients showed antibodies to gluten and 15% were overweight at onset of psoriasis.

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Pluripotent stem cell derivatives for full reconstruction of melanized pluristratifed epidermis

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Human epidermis has been produced in vitro for decades using adult epidermal stem cells from donors to provide cell therapy and industrial use in pharmaceutical and cosmetic applications. As potential alternative, pluripotent stem cells, immortal and pluripotent, have attracted major interest. However, available protocols do not allow producing melanocytes and keratinocytes capable of forming a fully differentiated epidermis out of human pluripotent stem cells. We present here a successful and robust protocol for that purpose, the success of which is based upon replication in vitro of the successive ontogenetic stages leading to formation of the epidermis. This protocol of differentiation generates first a homogenous population of keratinocytes and melanocytes with high proliferative capacity. Once seeded on an artificial matrix and either grown in vitro or else transplanted in immunodeficient mice, these cells form a melanised pluristratified epidermis that exhibits normal human characteristics. This opens the path for use of pluripotent stem cell lines in skin regenerative medicine and for industrial use.

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Dandruff is associated with changes in the density of Propionibacterium, Staphylococcus and Malassezia populations

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Dandruff is a scalp disorder occurring in about 17-50 % of human individuals depending on the population scrutinized, characterized by an abnormal flaking of the scalp. Although the presence of bacteria on the scalp has been known for a long time, previous reports have suggested that dandruff was only due to M. restricta and M. globosa with the exception of McGinley's paper published in 1976 who looked at the analysis of the bacterial and fungal communities on dandruff scalp. This last study was only based on culture and haemacytometer quantification, and the species M. restricta was not identified until 1996. In the present study, the diversity of bacterial and fungal communities of 19 scalp surface associated with dandruff was investigated using cloning and sequencing of the conserved ribosomal unit regions (16S for bacterial and 28S-ITS for fungal). Three major microbial species have been found in dandruff or non dandruff scalps: Propionibacterium acnes, Staphylococcus epidermidis and Malassezia restricta. Phylogenetic analysis of 824 16S rDNA sequences of S.epidermidis, 1005 sequences of P.acnes and 2000 ITS-28S rDNA sequences of M.restricta showed the presence of various phylotypes. However, none of the fungal or bacterial phylotypes were preferentially associated with dandruff since the same proportion of each phylotype was found in the dandruff and non dandruff groups. The three main species were further quantified with specific TaqMan MGB probes. The present data showed that in contrast to other studies, dandruff is not correlated to the higher incidence of one fungal species but rather to changes in the proportion of fungal and bacterial species on the scalp.

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Fumaric esters improve psoriasis via different gene signalling pathways than etanercept

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Fumaric acid esters (FAE) are an effective systemic oral treatment for psoriasis that is widely used in Europe. However, the mechanism of action by which FAE improve psoriasis is largely unknown. To identify involved pathways and mechanisms, Affymetrix gene arrays were used for analyzing gene expression profiles in psoriatic lesional skin from FAE-treated patients. At baseline and at 12 weeks of FAE treatment, RNA was isolated from lesional skin biopsies from 5 responders (> PASI-75 improvement) and 5 non-responders (< PASI-50). Gene expression was analyzed with Ingenuity Pathway Analysis (IPA). The gene expression pathways affected by FAE treatment were compared with pathways affected by etanercept (anti-TNFα) treatment (NCBI Gene Expression Omnibus GSE11903). Comparison of gene expression profiles at baseline and at week 12 in responders to FAE treatment revealed 169 probe sets differentially expressed (>2-fold change, adjusted $P<0.05$). FAE treatment most significantly affected the canonical pathway 'role of IL17A in psoriasis', reducing the expression of CCL20, CXCL1, DEFB4, S100A8 and S100A9. Comparison of responders and non-responders at week 12 revealed 471 differentially expressed probe sets, including SERPINB4 and DEFB4, which both showed >20 fold change upregulation in the non-responders. When comparing changes in gene expression of psoriasis-related molecules during FAE and etanercept treatment, we noticed minimal overlap, limited to the upregulation of FRZB, KRT15, S100A8 and S100A9. Despite similar clinical improvement, gene expression in lesional skin during FAE treatment shows little overlap with the expression profile during etanercept treatment. Effective treatment of psoriasis with FAE is firmly linked to suppression of 'the role of IL17A in psoriasis'. This is an obvious but novel clinical mechanism of action of FAE in psoriasis.

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Cutaneous Manifestations of Helicobacter Cinaedi Infection

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Helicobacter cinaedi (H. cinaedi) is a Gram-negative spiral bacillus that inhabits the intestinal tracts of mammals. It has been implicated as the cause of human gastroenteritis and bacteremia, particularly in immunocompromised individuals. Although cellulitis is sometimes reported to accompany infection by this pathogen, the cutaneous manifestations are poorly understood. To clarify the characteristic cutaneous features associated with H. cinaedi infection, 47 cases of H. cinaedi bacteremia experienced at one hospital as nosocomial infection were retrospectively evaluated. 34% (16 cases) of the H. cinaedi bacteremia patients showed cutaneous lesions. They all had sudden onset of erythemas accompanied by high temperature. The most common cutaneous manifestations of H. cinaedi bacteremia were found to be superficial cellulitis, which appear as painful erythemas or infiltrated erythematous plaques on the extremities. This study demonstrates these skin lesions can be an early clinical indicator of H. cinaedi bacteremia in the setting of nosocomial infection.

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Reflectance Confocal Microscopy: an effective tool for monitoring UVB phototherapy in psoriasis

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Background: In vivo reflectance confocal microscopy (RCM) is a novel, non-invasive imaging technique which enables imaging of skin at a cellular resolution comparable to conventional microscopy. Objectives: We performed a pilot study to evaluate RCM as a non-invasive tool for monitoring UVB phototherapy in psoriasis. Methods: In six patients with psoriasis, lesional and non-lesional skin was selected for RCM imaging using a standardized protocol. Well-known histological features of psoriasis were visualized: parakeratosis, acanthosis, agranulosis, papillomatosis, presence of epidermal inflammatory cells, increased number of papillary capillaries and increased capillary blood flow. RCM imaging was performed before the first irradiation with UVB-phototherapy, after nine irradiations, at clearance and 12 weeks after clearance. In four patients 4 mm punch biopsies were obtained and hematoxylin-eosin stained. Additionally, immunohistochemical staining was performed with monoclonal antibodies specific for CD31, CD3, Filaggrin, K16, Ki67 and CD1a for correlation to RCM images. Results: There was a high correlation between clinical, RCM and histological features. Normalization of RCM and histological features corresponded highly to clinical improvement of psoriasis. Conclusions: This study is the first to establish the use of RCM as an effective tool for non-invasive monitoring of UVB phototherapy in patients with psoriasis. Potentially, RCM could be used in many other skin diseases for monitoring therapeutic response on a cellular level in a clinical or research setting.

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Application of thiocyanate ion and peroxide as antimicrobial wound therapy

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Thiocyanate ion, a broad acting anti-bacterial agent, is catalytically converted to the active agent, hypothiocyanate by transfer of oxygen from hydrogen peroxide donor to the thiocyanate ion acceptor. A mouth wash with hydrogen peroxide and thiocyanate ion is effective in reducing mouth sores. Use of this modality to treat acute or chronic wounds has not been reported. Here we address several critical issues: 1) cytotoxicity of thiocyanate ion and hydrogen peroxide on keratinocytes, 2) antimicrobial effectiveness on bacteria that infect wounds, and 3) the choice of salivary peroxidase. A stock solution of 20mM potassium thiocyanate and 80mM hydrogen peroxide was freshly prepared and diluted twenty-fold in to ice-cold phosphate-buffer solution. The latter working solution was applied for 24 hours to serum-free cultures of normal human keratinocytes prewashed with ice-cold phosphate buffer immediately prior treatment. Control cultures received only the phosphate buffered solution. At the termination of the study, visual phase contrast microscopic observation showed no discernable difference between the test and controls. Further, standard cell counting methods revealed no cytotoxic loss of cells in test treated over or above the controls. Concentrations of thiocyanate for 24 hours up to 50mM were not toxic to keratinocytes. The in vitro cytotoxicity of hydrogen peroxide on keratinocytes was also conducted. Hydrogen peroxide in serum-free culture medium greater than 2mM inflicted minimal cell damage as seen by reduced uptake of crystal violet stain. These data suggest that basal layer keratinocytes may require lower hydrogen peroxide concentrations in the final formula. Antibacterial studies of the action of thiocyanate /hydrogen peroxide solutions were performed using standard microbiological agar plate assay against Pseudomonas aeruginosa as a pathogen often found in pressure ulcers. A five to six log reduction in number of P. aeruginosa was found. These studies confirm the feasibility of applying nontoxic concentrations of thiocyanate/hydrogen peroxide to treat infected cutaneous wounds.

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Novel Polymerase Chain Reaction Method for Detection of Cutaneous Human Papillomavirus DNA

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There was no simple method to identify the human papillomavirus (HPV) genotypes that cause cutaneous warts. A new polymerase chain reaction (PCR) method, called SK-PCR, was developed for this purpose. This PCR amplifies 210–238 base pairs of L1 DNA of 17 HPV types (HPV-1a, -2a, -3, -4, -7, -10, -27, -28, -29, -40, -57, -60, -63, -65, -77, -91, and -94), which are thought to cause various cutaneous warts, including common, flat, butcher's, punctate, and pigmented warts. The method is novel because the location of these primers is completely different from that of any previous PCR method for HPV. The target sequences are specific to alpha-, gamma-, and mu-papillomaviruses (PVs), but not to beta-PVs. Overall direct sequencing and restriction fragment length polymorphism (RFLP) were used to determine the HPV genotypes. Fifty of samples of plantar warts were examined, and HPV-27 was identified in 22 warts, HPV-57 in 15 warts, and HPV-2a in 9 warts. These PVs, which are alpha species 4, were the most common. HPV-4 and -65 (gamma-PVs) and HPV-1a and -63 (mu-PVs) were detected in one case each. A single HPV type was identified in all of these warts. This method appears to be useful for genotyping the HPVs causing cutaneous warts, and for distinguishing between HPV-induced warts and warty lesions unrelated to HPV infection.

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Safety and Efficacy of novel topical herb-based lotion (KAM-2306) to manage atopic dermatitis secondary infections

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Introduction: Atopic dermatitis (AD) is a common chronic inflammatory skin disease, characterized by eczematous lesions, xerosis and pruritus. In severe eczema secondary infections may predominate. The condition develops due to impaired skin barrier and altered immune reactivity. KAM-2306 is a barrier-based topical lotion augmented with plant extracts that stimulate antimicrobial peptide beta-defensin involved in innate immune defense. Purpose: To assess the safety/efficacy of KAM-2306. Methods: A cohort of 50 AD subjects were recruited to an open-label, non-randomized, interventional study. Thirty subjects were treated with KAM-2306 and 20 subjects were treated with Carrier Lotion for 21 days. The severity of AD was evaluated with SCORAD index at each visit (days 0, 7, 14 and 21). Results: The SCORAD index steadily decreased throughout the study, reaching 46% reduction at the final visit as compared to the baseline. All 6 intensity signs steadily decreased: the most prominent effect was recorded for oozing, associated with secondary infections, with an 86% decrease at the final visit in 26 out of 28 patients (93%). Pruritus was reduced by 60% at the final visit. In comparison to a Carrier Lotion, KAM-2306 demonstrated a stronger reduction in SCORAD after 21 days of treatment, particularly in symptoms related to secondary infections. KAM-2306 equally affected severe and mild/moderate AD patients, while effect of Carrier Lotion was reduced in severe AD patients. No secondary infections developed during the trial, and no adverse events were recorded. Conclusions: KAM-2306 is safe & effective for symptoms relief of mild/moderate and severe AD. By providing a mechanical barrier, the lotion maintains a moist skin environment, protects the skin from additional irritation and facilitates the healing process. The addition of herbal extracts stimulating beta-defensin expression, might reduce secondary infections symptoms and further protect the skin, thus contributing to the restoration of skin barrier's integrity.

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Serum YKL-40 as a possible biomarker for psoriatic arthritis

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YKL-40, also known as chitinase 3-like protein 1 (CHI3L1), is a chitinase-like protein which is lacking chitinase activity. Clinically, increased serum levels of YKL-40 have been reported in conditions with inflammation and/or tissue remodeling, such as rheumatoid arthritis, Crohn's disease and cancers. We have recently found that serum levels of YKL-40 are increased in psoriasis vulgaris (PV) and in generalized pustular psoriasis (GPP) and we proposed YKL-40 as a possible biomarker for those psoriatic disorders. In the present study, serum levels of YKL-40 in PV and GPP were compared with those in 18 cases with psoriatic arthritis (PsA), which were diagnosed based on the CASPAR criteria. Serum levels of YKL-40 were significantly increased in those cases with PsA and the mean YKL-40 concentration was higher than that in PV and was comparable with that in GPP. In 5 cases with PsA, the serum levels of YKL-40 were significantly improved by treatment with infliximab or adalimumab. Thus, serum levels of YKL-40 may also be useful as a biomarker for the treatment of PsA.

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Treatment algorithm of hidradenitis suppurativa / acne inversa

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Hidradenitis suppurativa / acne inversa (HS) is a chronic, recurrent, follicular skin disease usually presenting after puberty and been potentially scaring. It manifests with painful, deeply localized, inflammatory skin lesions that occur in apocrine gland-rich areas of the skin, most commonly in the axillae and the inguinal and anogenital regions (Dessauer definition). The recognized trigger factors include smoking and obesity. The treatment of HS is often disappointing, and has a significant negative impact on patients' quality of life. Regarding stressfulness and reduction of life quality HS ranks on the top among all dermatological diseases. There are no approved drugs for HS treatment. According to empirical analyses of treatment measures only topical 1% clindamycin solution, the oral systemic combination of clindamycin and rifampicin, the hormonal antiandrogen combination of ethinyl estradiol and cyproterone acetate, the biologics infliximab and adalimumab, and surgery reached an evidence level 2 and a grade B recommendation. To accomplish a grade-relevant treatment, the HS experts group of the German Dermatological Society (DDG) proposed the following treatment algorithm: Hurley's grade I: Topical 1% clindamycin solution treatment followed by systemic clindamycin 300 mg 2-3x/d (or minocycline 2x50 mg/d) and rifampicin 300 mg 2x/ d p.o. for 4-12 weeks, with clindamycin 300-600 mg 2-3x/d administered i.v. during the first 5 days of treatment. For women with signs of hyperandrogenism/ hyperandrogenemia oral antiandrogen hormonal therapy with ethinylestradiol/cyproterone acetate (up to 100 mg/d) should be administered. Hurley's grade II: Like in grade I followed by limited excision of recurrent lesions (alternatively ablation with the CO_2 laser). Hurley's grade III: Like in grade I followed either by wide excision or by infliximab infusion 5 mg/kg body weight once or twice (after one week) or adalimumab (160 mg s.c. and 80 mg one week later if required) to reduce the lesional and perilesional inflammation, followed by the wide excision of the involved area.

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Stimulating mitochondria activity in association with LED exhibits cumulative anti-aging benefits

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Light Emitted Diodes (LED) has been increasingly used in recent years as a potent physical anti aging effect. It has been demonstrated, in vitro, that LED acts by stimulating cell mitochondrial organelles and by up-regulating the cytochrome electron transport. In these studies, we were interested in finding synergies between LED and compounds applied into the skin, in order to enhance LED anti aging effect on skin. IV 10.005 and IV11.002 compounds, designed to stimulate respectively skin Extra-Cellular Matrix (ECM) or mitochondrial activity, were evaluated in combination with LED. Fibroblasts were exposed to 12J/cm2, twice a week, for one week. Biofunctionals were applied at 1% on fibroblasts, for 96 h for IV11.002 and 48 h for IV10.005. Although, the application of IV 10.005 biofunctional or LED, separately, significantly enhanced collagen1, collagen3 and fibronectin expression. Surprisingly, the cumulative treatments of both did not reveal a significant synergy on the modulation of these ECM proteins. In contrast, combining mitochondrial photomodulation and biomodulation with IV11.002, showed an cumulative effect with an enhanced expression of these ECM proteins. Consequently, in order to confirm these results, we performed a clinical double blind study on 10 volunteers. Volunteers' forearms were exposed twice a week during 28 days to LED 40J/cm2, and treated once a day with a cream containing IV11.002 or placebo. Four weeks later, in vivo confocal microscopy assessment and clinical self-evaluation demonstrated that the skin where LED was combined with the cream containing IV11.002 showed an cumulative rejuvenating effect. These results confirm that mitochondrion is a key target of LED, and that LED skin anti-aging benefits could be optimized by the association with topical application of biofunctionals that target mitochondria.

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Analysis of cell proliferation activity in human cutaneous tumors derived from keratinocyte by using immunohistochemistry-based Cell Cycle Detection (iCCD)

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Human cutaneous tumors derived from keratinocyte are supposed to occur with dysregulation of cell cycle. *P53* gene is a key regulator of cell cycle, and is known as a tumor suppressor gene. The mutation of *p53* gene is detected in majority of malignant cutaneous tumors derived from keratinocyte such as squamous cell carcinoma (SCC) and Bowen's disease (BD), and results in uncontrolled cellular proliferation of the tumors. However, no objective markers were developed for the evaluation of dysregulation of cell cycle. We analyzed cell proliferation activity in seborrheic keratosis (SK), actinic keratosis (AK), BD, and SCC by using a novel method, immunohistochemistry-based Cell Cycle Detection (iCCD), which is a multiplex immunohistochemical method that can simultaneously stain cells in the G1 and S/G2/M phases and those undergoing apoptosis with the 3 markers Cdt1, geminin, and gamma- H2AX. The results clearly indicate that most cells are in the G1 phase in normal epidermis and SKs. In contrast, AKs, BDs, and SCCs show high frequency of S/G2/M and apoptosis cells in the lesions, and also present distinct expression pattern of the markers from the adjacent skin, indicating the tumor margin. The present observations suggest that the iCCD can be one of the useful tools to differentiate the cutaneous malignant tumors from benign ones, and could be a practical method to decide the treatment plan for malignant cutaneous tumors.

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Intralesional Steroids and Keloid Disorder; Can They Make Keloids Worse?

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Introduction: Triamcinolone acetonide is widely used in treatment of keloids [1]. Patients' perceptions of the efficacy of intralesional steroids is presented with attention to worsening of keloids in 17.7% of patients and low overall rate of efficacy. Materials and Methods: An IRB approved online questionnaire was answered by unselected group of keloid patients[2]. From November 2011 to May 26, 2012, 261 patients completed the survey and 147 reported prior treatment with intralesional steroids. Results and Discussion: 65 (45.1%) patients reported 5 or less injections, 17 (11.8%) patients had between 6-9 injections and 60 (41.7%) patients had more than 10 injections. 5 patients could not recall number of injections. 2 patients (1.4%) reported cure of their keloids, 46 patients (31.3%) reported improvements, 73 patients (49.7%) reported no improvements and 27 patients (17.7%) reported worsening of their keloids Conclusion This study indicates that intralesional steroid injections are effective in only one third of patients; and can cause worsening of keloids in about 17% of patients. Although steroid injections are commonly used to treat keloids, the response rates to this treatment needs to be better defined. References: 1. Sexton GB, Local Injection of Triamcinolone Acetonide in the Management of Certain Skin Conditions, Preliminary Report, Can Med Assoc J. 1960; 83(26): 1379–1381. 2. www.KeloidSuvey.com.

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Quality Of Life is Worse Among Rheumatologist-Diagnosed Psoriatic Arthritis Patients Than Other Psoriasis Patients Seen in Dermatology Clinics: Results of the PREPARE Study

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The objective was to quantify differences in Quality of Life (QoL) between Psoriatic Arthritis (PsA) patients and psoriasis patients without arthritis seen in dermatology clinics in the PREPARE study (NCT01147874). Patients with plaque psoriasis were enrolled from 34 dermatology clinics in North America and Europe, regardless of psoriasis severity, joint symptoms or previous PsA diagnosis, and were evaluated by rheumatologists for PsA. Patients completed the Health Assessment Questionnaire (HAQ, scored 0=best, 3=worst, minimal clinically important difference [MCID]=0.30), the Dermatology Life Quality Index (DLQI, 0=no impairment, 30=worst impairment, MCID=5), the EuroQoL (EQ-5D, 0=death, 1=perfect health, MCID=0.05) utility questions and the EQ-5D Visual Analogue Scale (VAS, 0=worst health, 100=best health, MCID=5), and the Hospital Anxiety and Depression Screening (HADS, 0=no symptoms, 21=severe). Out of 949 patients who completed the study, 285 (30%) had PsA. PsA patients had statistically significant and clinically meaningfully worse HAQ, EQ-5D and EQ-5D VAS than other psoriasis patients (0.65 vs. 0.26, 0.66 vs. 0.80, and 66.8 vs. 76.5, p<0.001 each). DLQI scores for PsA patients were statistically worse, but the difference was not clinically meaningful (8.0 vs. 6.0, p<0.001). More patients with PsA had at least mild symptoms of anxiety and depression relative to those without (anxiety, 44.0 vs. 33.2%; depression, 29.1 vs. 19.7%, respectively, p=0.002). In conclusion, among psoriasis patients in this screening study, nearly one-third had PsA. PsA patients had worse QoL than plaque psoriasis patients. Dermatologists are in a unique position to screen for PsA and to ensure that patients with symptoms of PsA are evaluated by a rheumatologist and/or receive appropriate treatment for their joint disease.

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The PrecisePASI accurately reflects small changes of psoriasis disease activity

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Introduction: The Psoriasis Area and Severity Index is the score of choice to grade severity of psoriasis and detect clinical changes over time. However, as our treatment options improve and patients' expectations rise, the PASI scores often are in the range below 10. It has been repeatedly shown in the range of 0-10, the PASI score is neither sensitive nor very reproducible. This is due to the low resolution of the area classes of 0-6 that were originally used to enable calculation of the score by hand. Here we sought to overcome this problem within the bounds of the original PASI. Methods: The PrecisePASI grades erythema, scaling and infiltration with 0-4 in head/neck, trunk, arms and legs. The area involvement however is registered in actual percentages instead of the area classes. Congruent with the original PASI's discontinuous area curve, each percent area below 10% is graded 0.2 points and 0.05 points above 10%. Each area score is then weighted and summed up as in the PASI. Results The PrecisePASI has a linear increase while the PASI has a staircase-pattern when the change in area involvement is continuously increased. Both scores meet at the endpoint-relevant values of 10% and at the PASI area-class defining percentages 30, 50, 70 and above. 768 patients were analysed with both PASI and PrecisePASI. In the region of BSA 2-10, the both scores matched closely (mean 0.13 +/- 0.72), but in the region of BSA < 2%, the PASI was up to 4.76 higher than the PrecisePASI, mean 0.94 (+/- 0.78). Above BSA 10% instead, the PASI was up to 6.08 lower than the calculation with actual percentages (mean 1.12 +/- 1.10). Conclusion The PrecisePASI is an advanced calculation but not adaptation of the original PASI score. It corrects the undesired inaccuracies of the PASI in the lower BSA ranges, is backward compatible with the original PASI and is a tool to use as an endpoint in trials aiming to detect differences in the lower ranges of BSA.

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The correlation between clinical diagnosis and punch biopsy in typing basal cell carcinoma

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Skin cancer is the most common of all malignancies and its incidence continues to rise. This rising incidence leads to a substantial burden of disease in both patients and the health care system. To be able to continue to provide sufficient care for all patients, different management strategies are necessary. One is to explore the possibility of avoiding punch biopsy in diagnosis BCC (basal cell carcinoma) and SCC (squamous cell carcinoma) by trusting in the clinical diagnosis. Our objective was to investigate the correlation between clinical diagnosis and punch biopsy in diagnosing BCC and SCC as well as the correlation between clinical diagnosis compared to punch biopsy in typing BCC. 100 patients with 124 suspect lesions for BCC or SCC were included and underwent a 3 mm punch biopsy. Furthermore a short medical questionnaire was completed by . Clinical diagnosis was compared to histopathological diagnosis of punch biopsy. We included 100 patients with 124 clinically suspect lesions. PPV (positive predictive value) of clinical diagnosis for BCC was 56.3%, in typing BCC for sBCC (superficial BCC) 43.8%, for nBCC (nodular BCC) 34.5% and for iBCC (infiltrative BCC) 38.5%. PPV of clinical diagnosis for SCC was 23.8%. We found that 12.9% (N=16) of all included lesions were underestimated of which 2 cases of clinical suspect sBCCs and 3 cases of clinical suspect nBCCs appeared to be SCC. In contrast, 50.8% (N=63) were overestimated by clinical diagnosis whereof 43.5% was not even malignant. However, 14 of the 32 clinical suspect sBCC, were actually sBCC, 12 lesions were actinic keratoses and 3 were morbus Bowen (total 90.6%, N = 29), all requiring the same therapy. Our results showed that passing puch biopsy in suspect nBCC and iBCC would result in mistreatment.

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Profiles of patients with psoriasis associated with hepatitis C virus infection. A case control study

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Psoriasis is a chronic inflammatory skin disease with various complications such as arthritis, diabetes mellitus, and hypertension. Hepatitis C is a chronic infection of hepatitis C virus (HCV), eventually leading to liver cirrhosis and hepatocellular carcinoma. The association of psoriasis and HCV has been reported but there have not been a detailed case control study yet. To outline the profiles of HCV positive psoriatic patients, patients with diagnosis of psoriasis who visited Fukuoka University from 1991 to 2011 were sought in the database and their medical records were manually checked for detailed information about history of hepatitis C, or serum anti-HCV antibody (Ab). Anti-HCV Ab positive (HCV+) patients were compared to a control group (HCV-). There were 54 (7.5%) HCV+ patients among 717 psoriatic patients. HCV+ patients had stronger male predominance (male:female=44:10 vs control 438:225, p=0.023), later onset (median age, 55 vs control 41, p<0.0001), lower body mass index (22.41 vs control 24.12, p=0.0126), more frequent association of diabetes mellitus (DM+/-=16/31 vs control 102/464, p=0.0116). HCV infection preceded onset of psoriasis decisively in 89%. Interferon therapy exacerbated 70% of pre-existing psoriasis, and induced psoriasis de novo in 8 patients. Our observation revealed that HCV+ psoriatic patients have less BMI and more diabetec than HCV- patients do. This is possibly because HCV infection causes chronic inflammation and overproduces TNF-alpha, which induces psoriasis in some patients with certain predisposition. HCV infection can be an inducing factor of late onset psoriasis.

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Unresponsiveness to etanercept: is there a common phenotipical profile of patients?

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Etanercept is a tumor necrosis factor-alpha antagonist whose efficacy and safety in the treatment of psoriasis have been mainly proven. Nevertheless, few studies evaluate a prior or secondary inadequate response. Our study aimed to evaluate the unresponsiveness to etanercept in daily clinical practice for the treatment of psoriasis to eventually outline a common phenotipical profile of patients. Between June of 2003 and December of 2011 a total of 605 psoriatic patients underwent etanercept in our center. We carried out a retrospective observational study that collected data on 33 patients, affected by moderate-to-severe plaque type psoriasis (24) and psoriatic arthritis (14), treated continuously with etanercept 50 mg twice weekly for 12 weeks and subsequentely 50 mg once weekly, or etanercept 50 mg weekly, respectively. The following variables were evaluated: patients' morphologic characteristics, comorbidities, previous performed treatments, outcome of the psoriasis area severity index (PASI) [PASI 50, PASI 75, and PASI 90] and joint pain. Unresponsiveness to etanercept was evaluate by PASI score unimprovement from baseline. In particular, primary inefficacy was the proportion of patients not achieving a reduction in PASI score >50% at week 12 as compared to baseline, on the contrary secondary inefficacy is the lost of these achievement during the treatment. In our study, at baseline mean PASI score was 11.47 (range: 2-28.4). At week 12, 9/33 patients (27.27%) achieved a PASI reduction from baseline <50% (primary inefficacy), while 24/33 patients (72.73%) achieved a PASI reduction from baseline >50% and subsequently loosing efficacy during etanercept therapy (secondary inefficacy). The mean treatment duration was 106.49 weeks (range: 12-240). Our study demonstrate that most of the patients obtain the response in the short-term treatment with etanercept, although a decrease in efficacy is observed; meaning that secondary inefficacy is more frequently observed than the primary one.

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Change in the echogenicity of the dermis caused by change of hydration monitored using high frequency ultrasound

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Hydration is one of the most important parameters in assessing the appearance and condition of skin. So far, measurements of skin hydration were performed using tewameters or corneometers. However, whereas several difficulties in performing such measurements, it is important to look for other methods that would enable assessment of the skin hydration degree. The aim of this study was to evaluate the use of high frequency ultrasound to determine the level of skin hydration based on the echogenicity of the dermis. The sampling consisted of 27 women aged 20-67 years with dry skin on the lower extremities. The women were subjected 14 days of therapy consisting of the use of preparations for dry skin, ie cleansing gel and body butter. The following parameters were evaluated: thickness of the epidermis, dermis thickness, echogenicity of the dermis. In addition hydration, lubrication and transepidermal water loss (TEWL) were measured on the skin surface. As a result of therapy skin's appearance has improved. Change in the appearance of the skin resulted in noticeable changes in ultrasonography image. There were statistically significant differences in the echogenicity of the upper layer of the dermis. After the therapy echogenicity decreased. There were no statistically significant differences in the echogenicity of the lower layer of the dermis and in the thickness of the epidermis and dermis. In conclusion, high-frequency ultrasound enables to monitor changes in the skin associated with water storage base on changes in the skin echogenicity.

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A Maximal Use, Systemic Exposure Study to Assess the Safety, Tolerability, and Pharmacokinetic Profile of AN2728 Ointment, 2% in Subjects with Plaque Type Psoriasis

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The objective of this multicenter, open label, pharmacokinetic (PK) study was to evaluate the systemic exposure of AN2728 Ointment, 2% under maximal use conditions in subjects ≥15 y of age with plaque type psoriasis involving ≥25% body surface area (BSA), excluding the scalp. AN2728 was applied to all non-scalp psoriatic skin twice daily for 8 days (except Days 1 and 8 when a single morning dose was applied). Blood was drawn for PK analysis on Days 1 and 8, pre-dose samples on Days 2-8, and 24 h post-dose on Day 9. Urine samples were collected for PK analysis on Days 1 and 8. Safety evaluations included adverse events (AEs), vital signs, safety labs and 12-lead ECGs. Local tolerability symptoms of burning/stinging and pruritus were also assessed. The 33 enrolled patients had a mean age of 46 y and were 88% male. Baseline psoriasis involvement ranged from 25% to 80% BSA (mean = 38%) and Physicians' Global Assessment severity ranged from 2 (mild) to 5 (very severe) (mean = 3.3). No deaths, SAEs, or discontinuations due to AEs were reported during the study. A total of 11 AEs were reported by 7 subjects. All AEs were mild (64%) or moderate (36%); none was severe. Most (64%) AEs were unrelated or unlikely related to study drug. Mean scores for burning/stinging and pruritus improved substantially from Baseline and remained stable and low during treatment. AN2728 was rapidly absorbed with a median Tmax value of 1 h on Day 1 and 2 h on Day 8. Mean Day 1 Cmax and AUC(0-12) values were 167 ng/mL and 878 ng.h/mL, respectively. Corresponding mean Day 8 values were 109 ng/mL and 748 ng.h/mL. Overall, plasma exposure increased as the amount applied increased. There was no evidence of accumulation over 8 days of dosing. Steady-state was achieved within 3 to 4 days. No correlation was seen between incidence of AEs and higher plasma exposure levels. Based on these results, AN2728 Ointment, 2% applied to very large areas of psoriatic skin appears to be safe and well tolerated.

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Decreased stratum corneum hydration in type 2 diabetes patients is affected by blood glucose level, and a novel moisturizer containing physiologic lipid granules can improve barrier homeostasis as well as dry skin in diabetic patients

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Type 2 diabetes mellitus (DM) induces many skin problems related to chronic impaired skin barrier state. Recently, we reported that a long-standing hyperglycemia decreased stratum corneum(SC) hydration and delayed skin barrier homeostasis in Otsuka Long-Evans Tokushima Fatty rats, which correlated with HbA1c levels presenting recent blood glucose control. Also, in this animal model decreased epidermal lipid synthesis accounted for decreased lamellar body(LB) production. Therefore, we hypothesized that physiologic lipid granules including ceramide, fatty acids and cholesterol to mimic LBs with multiple lamellar structures have superior affinity to diabetic skin; better moisturizing effect. Patients with type 2 DM (n = 43, aged 40-86 years, HbA1c over 7) were recruited and instructed to apply the moisturizers with or without physiologic lipid granules on each extremity separately for 4 weeks. Before and after application, SC hydration, basal transepidermal water loss (TEWL) and barrier recovery rates were measured. Thirty-nine patients completed the study. SC hydration was significantly decreased in higher HbA1c (over 8.5) group compared to lower group and increased significantly by moisturizer with physiologic lipid granules in both groups. Basal TEWL was not different between both groups and not changed by application. Barrier recovery after tape stripping was significantly faster in moisturizer with physiologic lipid granules, but no difference between both HbA1c groups. There were no remarkable side effects related with moisturizer. In conclusion, SC hydration defining dry skin is much influenced by blood glucose control (HbA1c level) in type 2 DM patients, which is significantly improved by a novel moisturizer containing physiologic lipid granules.

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Comparison of the cutaneous inflammatory cell infiltrate in early and late-onset psoriasis

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In 1985, Henseler and Christophers classified psoriasis into early-onset (EOP) and late-onset (LOP). Genetic and immunological differences exist between EOP and LOP; HLA-Cw*0602 allele occurs more frequently (55-80%) in EOP than LOP (15-20%) and epidermal Langerhans' cells migration is different. To further investigate these differences, we assessed the histology and immune infiltrate in involved (PP) and uninvolved (PN) skin of both types. 32 patients aged ≥50y were recruited; 17 EOP (14 male, mean age 58±9y; 3 female, mean age 59±9y) and 14 LOP (8 male, mean age 66±5y; 6 female, mean age 62±6y) with a mean age of onset of 21 ± 8y and 54 ± 8y respectively. Skin biopsies were stained with H&E and antibodies against CD3, CD4 and CD8. Positive epidermal cells were counted per field at 200X magnification. Dermal infiltrate was assessed using a semi-quantitative (0-3) scale. A significantly higher count of epidermal CD3+ and CD4+ cells was noted in PP of LOP as compared to EOP patients; mean epidermal CD3+ in LOP was 42.8 ± 13.3 vs 31.7 ± 17.5 in EOP (p=0.05), mean epidermal CD4+ in LOP was 15.1 ± 6.2 vs 6.7 ± 4.6 in EOP (p=0.001). There was no significant difference in epidermal CD8+ counts in PP between the groups; mean CD8+ in EOP was 19.1±11.1 vs 15.8 ± 7.8 in LOP (p=0.33). The epidermal CD4+/CD8+ ratio of 1.3 in LOP was significantly higher compared to 0.5 for EOP (p=0.002). After correction for confounding variables, using linear regression, these remained statistically significant. No difference was noted in epidermal CD3+, CD4+ and CD8+ counts in PN between EOP and LOP. Neither dermal CD3+, CD4+ and CD8+ counts nor H&E staining were significantly different in PP and PN of both groups. The data indicate that EOP is characterized by a relative paucity of CD4+ cells and a predominance of CD8+ cells in the epidermis whereas LOP is characterised by an epidermotropism of CD4+ cells. Collectively, these data provide further evidence that EOP and LOP are different conditions.

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Histological response due to biologics treatment in psoriatic plaques

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Psoriasis treatments by biologics, such as TNF- α inhibitors and anti-IL-12/23 p40 antibody, have resulted in significant clinical benefits for patients. However, there are few reports about the mechanisms of biologics in lesional tissue. The aim of the present study is to evaluate the histological changes after administration of adalimumab (Humira), a TNF- α inhibitor, or ustekinumab (Stelara), anti-IL-12/23 p40 antibody in lesion of patients with plaque type psoriasis. Skin biopsy specimen were analyzed before and after treatment at 1 and 3 months from 4 Japanese psoriasis patients (three patients were treated by adalimumab and one patient was treated by ustekinumab) by hematoxylin and eosin (H&E) stain, immunohistochemistry and electron microscopy. The histological changes were evaluated to correlate with the disease severity assessed by Psoriasis Area and Severity Index (PASI) score. The lesional skin showed decreased epidermal thickness, orthokeratosis and decreased dermal inflammatory cells. Loricrin expression, a keratinocyte terminal differentiation marker, was restored and keratin 17, an aberrant differentiation marker was decreased. CD3, CD4 and CD8+ T lymphocytes were decreased in dermis, as well as CD11c+ dermal myeloid dendritic cells, and CD68+ and CD14+ macrophages. TNF+ cells were decreased, while IL-17A+ cells were increased in 2 cases treated by adalimumab. Electron microscopy revealed keratohyalin granules in stratum granulosum reappeared at 1 month. Biologics for psoriasis rapidly decreased dermal immunocytes, and epidermis was restored to normal differentiation. Decreased TNF+ cells may be a good marker of efficacies of biologics.

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Childhood Lichen Sclerosus is poorly recognised

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Childhood lichen sclerosus (LS) is a relatively rare, autoimmune skin disease. We focused on accuracy of diagnosis and features of childhood LS patients treated at Tampere University hospital in 1982-2010. The study comprised a register study, and a questionnaire to the patients. A search was performed in hospital patient record database using ICD codes for LS. All hits were reviewed. The data was picked up from patient records using a structured protocol. The patients also received a questionnaire dealing their symptoms and effect of LS on QoL. Altogether 45 children were treated for LS at Tampere university hospital in that time period, 44 girls and one boy. We focused on girls. The age at the onset of LS was mean 7.1 years (range 2-18), and only in some girls the disease was burst out after their menarche. The delay from onset of the symptoms to diagnosis was mean 1.3 years (range 0-8). Our estimation on prevalence was 0.09%. The majority of patients (84 %) were misdiagnosed or without diagnosis in the referring unit. A false suspicion of sexual abuse was expressed in one referral, and two suspicions arouse at the university hospital. Further features included another autoimmune disease in 6, Turner's syndrome in 2, kidney disease in 2, LS in family in 4. The majority were treated with topical corticosteroids, despite 8 developed architectural vulval changes, but no malignancies. Of 15 responding to questionnaire 9 (60%) had no follow-up, and 3/5 patients, who were asymptomatic in their final visit reported recurrence of symptoms. Nine girls reported lowered QoL. The association of LS and other autoimmune diseases should be recognized. The high prevalence of Turner's syndrome raises a question of the influence of oestrogen. The association with kidney diseases requires further research. Long-term follow-up is needed, because the cancer risk remains unsolved. Sexual abuse must also be considered, because LS may occur at the site of trauma.

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A "healthy, low-calorie diet" session for the treatment of recalcitrant atopic dermatitis

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A substantial portion of atopic dermatitis (AD) patients treated with conventional therapies become intractable to treatment after experiencing several cycles of recurrence. Among these cases, a specific group of AD patients are found to consume an excessive amount of n-6 fatty acids and/or sugar. We have achieved good clinical outcomes in such patients by adding dietary instructions to conventional therapies. When symptoms remain intractable in spite of usual treatment with topical steroids and anti-histamines, the patients are advised to join a session we call, "Refresh in the hospital!", which is intended to help them avoid fatigue, while also teaching them how to consume a healthy diet. SCORAD (SCORing Atopic Dermatitis) and QOL scores of seventy-seven patients (age: 4-75 (30±13, mean±SD); M:34, F:43) admitted (for two to 20 (9±4, mean±SD) days) for recalcitrant AD from April 2008 to March 2012 were examined and compared with those of outpatients. During hospitalization, the adult patients consumed approximately 1600 kcal/day of rice, boiled vegetables/beans and seafood, similar to the "traditional Japanese diet" consumed in Japan until the 1960s. The SCORAD scores of the patients decreased significantly from 51±20 (mean±SD) to 17±12 after hospitalization and continued to decrease one month (20±14), three months (18±11) and three years (17±10) after discharge. Although the scores of the outpatients measured at three years (16±16) also decreased from baseline (46±20), the scores of the outpatients measured at one (33±21) and three months (26±15) were significantly higher than those of the inpatients ($p<0.01$, 0.05). The QOL scores of the inpatients also decreased significantly after hospitalization. For patients with recalcitrant AD, hospitalization to experience a healthy diet with 30% fewer calories improves SCORAD and QOL scores. Such behavioral/cognitive sessions may complement and improve the long-term effects of standard medical therapies.

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A retrospective analysis of clinical and immunological parameters in pemphigus patients

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Pemphigus is a potentially life-threatening blistering autoimmune disease remaining therapeutically challenging. A commonly applied therapy consists of high-dose systemic corticosteroids in combination with immunosuppressive agents such as azathioprine or mycophenolate mofetil. In therapy-resistant patients second line therapies such as immunoadsorption, the B-cell depleting anti-CD20-antibody rituximab or high dose intravenous immunoglobulins are needed. In this retrospective study we investigated the therapeutical response of first and second line treatment options on the basis of clinical and immunological parameters of 20 pemphigus patients refractory to combined systemic immunosuppressive therapy. The median observation period was 42 months [range: 6 to 72 months]. During this observation period, clinical activity quantified by the recently introduced ABSIS score as well as immunological parameters such as anti-desmoglein 1 and 3 autoantibody titers were determined regularly. Two patients received systemic immunosuppressive therapy only; another 2 patients received adjuvant rituximab or immunoadsorption, respectively, while 14 patients received immunoadsorption in combination with rituximab and 3 of those were treated additionally with intravenous immunoglobulins. During the observation period, antibody titers correlated well with the clinical activity in most of the cases. After a median observation period of 42 months, 11 of the 20 patients were in complete remission, 6 of them without any immunosuppressive treatment. In 8 patients a partial remission has been achived and 1 patient still showed a high clinical activity. Five patients relapsed after combined treatment with immunoadsorption and rituximab and received another cycle of IA and Rtx again. The present findings show that in severe and refractory pemphigus patients the standard therapy is often not sufficient. In these cases, with second line therapies a long term control of the disease activity can often be achieved.

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Intermediate to long-term efficacy and safety of etanercept: report from the psoriasis registry Austria

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More information is desirable on the anti-psoriatic long-term efficacy of biologics, including etanercept. We analyzed available data on the clinical efficacy of etanercept in patients with chronic plaque psoriasis treated under daily life conditions at the Department of Dermatology, Medical University of Graz, Austria. Data from the psoriasis registry Austria (http://www.psoriasisregistry.at) of 77 adult psoriasis patients (29 women and 48 men; median age, 49 years; age range, 15 to 79 years) with median disease duration of 19 years (range, 1 to 49 years) treated with etanercept between 2005 and 2012 were extracted for analysis. The efficacy endpoints of this retrospective study were per-protocol complete remission (CR), PASI 90, PASI 75, and PASI 50 reduction. Seventy-two patients completed 3 months of treatment, 70 completed 4 to 6 months, 60 completed 7 to 12 months, 44 completed 13 to 24 months, 33 completed 25 to 36 months, 20 completed 37 to 48 months, 7 completed 49 to 60 months, and 2 completed 61 to 72 months of therapy. Thirty-six of 77 (47%) patients discontinued etanercept-treatment, including 9 (12%) due to lack of efficacy, 8 (10%) due to side effects (2, injection site reactions; 2, soft tissue infection; and 4 others), 7 (9%) due to switch to another treatment, 6 (8%) due to patient wish, 5 (6%) due to satisfactory therapeutic response (at least PASI 75), and 1 (1%) due to malignancy (bladder cancer). The CR, PASI 90, PASI 75 and PASI 50 rates were 2%, 20%, 28% and 74% at month 3 (n, 46 patients); and 18%, 39%, 71% and 89%, at month 6 (n, 38 patients), respectively. Intermediate to long-term follow-up of patients with ongoing treatment of at least 6 months duration revealed that the CR, PASI 90, PASI 75 and PASI 50 rates were 8%, 46%, 81% and 97% after a median time of 34 months (range, 6 to 76; n, 37 patients). This analysis indicates that approximately 45 to 50% of psoriasis patients exhibit a favorable long-term response to treatment with etanercept.

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Analysis allergic reactions in two patients with anaphylaxis induced by the ingestion of shiitake mushroom

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Shiitake are popular edible mushrooms all over the world, and eating raw shiitake may lead to relatively common 'shiitake dermatitis' or toxicodermia. There are few reports of immediate allergy from shiitake mushroom. We report two cases of anaphylaxis that occurred immediately following the ingestion or touching of shiitake mushroom. However shiitake mushroom allergen which has so far been identified and characterized. The aim of our study was to analysis allergic reactions in two patients with anaphylaxis induced by the ingestion of shiitake mushroom. The two patients were 42 year-old, female office worker (case 1) and 35 year-old, male shiitake grower (case 2). Serum samples were collected from two patients. We examined serum IgE level in these patients. Next, extracts from shiitake mushroom were analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), Western blotting and mass spectrometry. The two patients have normal IgE levels (case 1: 32.4 IU/ml, case 2 : 136.0 IU/ml). Skin prick tests showed no positive reaction, but scratch testing were strongly positive for shiitake extracts in both patients. Western blotting revealed that the sera of both patients showed specific IgE binding to shiitake extracts, and no binding to normal serum. In conclusion, shiitake may cause immediate IgE-mediated allergy that is manifested in the skin as urticaria. This skin symptom is different from 'shiitake dermatitis' that is caused by eating raw shiitake. Skin tests and immunoblot for immediate allergy and detection of specific IgE are important when contact dermatitis from shiitake is investigated.

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Several types of clones existed at the same time in a patient with peripheral T-cell lymphoma, not otherwise specified

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Peripheral T-cell lymphoma, not otherwise specified (PTCL-NOS) has heterogeneous clinical histologic immunophenotypic, cytogenetic and molecular features, and it does not correspond to any of the specifically defined types of mature T-cell lymphomas in the current WHO classification. We present a case of a patient with PTCL-NOS who had three types of skin lesions. Clinically these lesions were plaque, tumor and subcutaneous tumor, and their histological findings were similar to mycosis fungoides, extranodal NK/T-cell lymphoma, nasal type and subcutaneous panniculitis-like T-cell lymphoma, respectively. Infiltrating tumor cells had CD3+CD4+CD56- phenotype and they were negative for Epstein-Barr virus. We extracted genomic DNA from three types of lesions and examined them with PCR-SSCP analysis based on BIOMED-2 methods. Different clonal tumor cells were detected from each lesion, and one of them had a clonal T cell receptor delta rearrangement. In addition we found three types of lesions repeatedly and they occurred at random. This result indicated that several types of clonal tumor cells existed at the same time in one patient with PTCL-NOS and that these different tumor cells made different lesions clinically and histologically. We suggest heterogeneous features of PTCL-NOS would be caused not only by instability of tumor cells but also by co-existence of different types of tumor cells.

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Transdermal drug delivery facilitated using a combination of microneedles and a magnetophoretic patch

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This study explores the use of a magnetophoretic drug patch in combination with microneedles to deliver fluorescein through the stratum corneum Microneedles and drug patches have been combined in the field of transdermal drug delivery and this has been coined the "poke and patch" technique. Microneedles are applied for a few seconds to the skin followed immediately by the application of a drug containing patch. Patch delivery of drugs is generally most successful with drugs of molecular weight less than 500 dalton The patch was applied to ex vivo abdominoplasty skin immediately following microneedle application .Transepidermal water loss (TEWL) was measured before and after microneedle insertion. This combination of microneedle and magnetophoretic patch was compared to drug delivery after microneedle insertion alone and compared to microneedle insertion followed by application of a passive,non magnetic patch . The substance chosen to assess transdermal delivery was sodium fluorescein, molecular weight 376. Penetration of fluorescein was assessed first with dermatosopic images to localise microneddle pores followed by laser scanning confocal microscopy (LSCM) in fluorescent mode:a z-stack of images were used to quantify fluorescence intensity and dye positive areas The results show the transdermal delivery of fluorescein was significantly increased over the passive control patch and delivery using microneedles without a patch

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Human papillomavirus in non-melanoma skin cancer

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HPV has been implicated along with ultraviolet radiation as a co-factor in non-melanoma skin carcinogenesis. In an ongoing case/control study we have been studying HPV in pre-cancerous skin lesions- actinic keratoses (AKs), squamous cell carcinoma (SCC)-in situ (CIS) and penile pre-cancer (PIN) as well as non-melanoma skin cancer (NMSC)-basal cell carcinoma (BCC) and SCC in HIV +ve and HIV –ve individuals. We have previously shown high rates of genital HPV types in both HIV + and HIV –ve non-genital NMSCs, with beta and next cutaneous types the most prevalent in the HIV +ve group. This work extends our HPV typing of HIV-ve NMSCs. A further 25 lesions from 8 HIV –ve individuals have been studied. DNA has been extracted from microdissected, formalin-fixed, paraffin-embedded tissue and typed for beta, genital and cutaneous HPV by broad-spectrum, highly sensitive assays.Total typed to date: 33 HIV –ve patients and 54 lesions (33 BCC, 14 SCC, 1 CIS, 5 AKs, 1 PIN). Genital types were detected in 11/21 (52%) patients +ve for any HPV type (4 BCC, 6 SCC, 2 AK: HPV 6, 16, 18, 52, 74); beta types in 8/21 (38%: 6 BCC, 1 SCC, 2 AK: HPV 8, 15, 22, 23, 24, 25, 36, 76, 80, 92, 93, 96); cutaneous types in 7/21 (33%: 2 BCC, 4 SCC, 1 PIN: HPV 1, 3, 4, 27, 29). Multiple types were identified in 5/21 (24%) HIV-ve individuals compared with 5/12 (42%) of HPV +ve/HIV +ve individuals previously reported. In summary, HPV is highly prevalent in pre-cancerous lesions and NMSC of HIV –ve and HIV +ve patients. Although isolated from non-genital skin, genital HPV types, including high-risk oncogenic types 16 and 18, are the most common. Beta types, reportedly the most prevalent HPV type in NMSC, are less common. Multiple types are less prevalent in the HIV –ve group, possibly reflecting differences in immune status.

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Efficacy of sodium L-ascorbyl-2-phosphate 5% lotion in the treatment of acne vulgaris *in vitro* and *in vivo*

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We recently conducted an open label clinical study and an *in vitro* study on inflammatory cytokine index, by using a preparation which contains 5% sodium L-ascorbyl-2-phosphate (APS) in commonly used general lotion, in order to research efficacy in the treatment of acne vulgaris (acne). In the open label clinical study, patients (17 to 32 years of age) with moderate acne lesions were enrolled and randomized to apply either APS 5% lotion (VCL) or placebo lotion (PL). Participating patients had not used isotretinoin for 6 months, or any other oral or topical agents for the treatment of acne for 3 months prior to entering the study. Forty-two patients completed the study protocol (22 in the VCL group and 20 in the PL group). Efficacy and adverse effects were assessed at baseline, and at weeks 4, 8, and 12, according to the standard assessment method by a specialist. Neither VCL nor PL group had any remarkable adverse effects. In the global improvement assessment, the percentage of patients in the VCL treatment group showing either good or excellent improvement was 77.3% ($p<0.05$) compared with 25% in the PL treatment group. In the inflammatory cytokine index study, after incubating for a period of 48 hours at 90-100% confluency, neonatal human keratinocytes (NHEK(F)) were treated for 4 hours with VCL. Peptidoglycan (PGN) from Staphylococcus aureus at a final concentration of 0.1 mg/mL was then added and cultured further. Interleukin (IL)-8 was measured in order to evaluate anti-inflammatory effects of VCL. Twenty-four hours after the stimulation with PGN, the amount of IL-8 in the culture supernatant fluid was measured by ELISA and compared. While treated with VCL, and 24 hours of a PGN-induced stimulation, the production of IL-8 was significantly inhibited in the culture medium in a dose-dependent manner (range; 0.63mg/mL - 2.5mg/mL). In addition to our past reports on the *in vitro* anti-inflammatory effect, this study suggests clinical efficacy of VCL for the treatment of acne as well as its inhibitory effect against inflammatory cytokines production.

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The clinical and histological effect of home-use, combination blue-red LED phototherapy for mild to moderate acne vulgaris in Korean patients: a randomized, controlled trial

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Standard acne medications have demonstrated relatively modest efficacy, but only at the expense of various side effects and discomforts. Therefore, there is a growing demand for an alternative treatment modality. This study was designed to determine the clinical and histological effects of the home-use, combination blue-red LED phototherapy (OCimple Light Therapy System MP 200, CERAGEM MEDISYS). A total of 35 patients with mild to moderate acne were randomly assigned to either blue-red LED device irradiation group or control group using sham device. Patients allocated to the treatment group were instructed to use LED device, twice per day for 4 weeks, serially receiving 420 nm blue light (0.91 J/cm2, 2.5 minutes) and 660 nm red light (1.22 J/cm2, 2.5 minutes) per session. Patients' follow up was conducted at 2, 4, 8, and 12 weeks after baseline. Skin biopsy was also performed for immunohistochemical analysis of MMP-9, TNF-α, IL-8, TLR-2, SREBP-1 and NF-κB. Our study showed that treatment group has shown significant clinical improvement in both inflammatory and non-inflammatory acne lesions after 2 weeks of treatment compared with control group, and the clinical efficacy maintained until 12-week follow up. Acne severity evaluated by both dermatologist and patients' self-assessments showed the similar patterns, and side effects were minimal. Histopathologic results were well correlated with clinical results, partly elucidating molecular mechanisms related with therapeutic effects. Therefore, our data confirm that combination blue-red LED phototherapy could be an alternative treatment modality for acne with remarkable efficacy and safety.

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First experience with adalimumab for two variants of linear IgA disease (LAD): classic LAD and IgA-mediated epidermolysis bullosa acquisita

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Linear IgA disease (LAD) is an auto-immune mediated subepidermal blistering disease occurring on the mucous membranes and the skin. In classic LAD, blistering occurs due to the fact that several antigens located above the sublamina densa are targeted by IgA, in which LAD-1 is the most important antigen. IgA-mediated epidermolysis bullosa acquisita (IgA-EBA) is another subtype of LAD. In IgA-EBA type VII collagen is targeted by IgA, which is part of the anchoring fibrils that are located in the sublamina dense zone. Both diseases are frequently resistant to conventional therapies with prednisolone, dapsone and colchicine. We treated two female patients with adalimumab who were diagnosed with respectively classic LAD and IgA-EBA, and failed on conventional treatment with dapsone. In both patients treatment with dapsone was combined with adalimumab. The classic LAD patient only mildly improved with 40 mg adalimumab twice weekly, however, pruritus and skin lesions improved significantly after increasing the frequency of administration to once weekly. Due to the development of an interstitial lung disease that was possibly induced by adalimumab, the therapy had to be discontinued. Within several weeks the patient had an exacerbation of LAD. The second patient, who was diagnosed with IgA-EBA, also had significant improvement of pruritus during treatment with adalimumab and the number of bullae declined within several weeks after commencement of adalimumab. However, after 6 months she had a relapse of symptoms and adalimumab was discontinued. Currently, her IgA-EBA is relatively stable on treatment with dapsone, which is periodically combined with prednisolone. These cases illustrate that the TNF-α inhibitor adalimumab may be effective in both subtypes of LAD when conventional treatment fails. However, studies are needed to determine the role of TNF-α in the pathogenesis of both subtypes of LAD, the effectiveness of adalimumab and to establish appropriate dosing schedules.

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Hidradenitis Suppurativa: The Effect Of Combinated Treatment With Oral Clindamycin And Rifampicin

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Hydradenitis Suppurativa (HS) is a chronic disease with exacerbations characterized by inflammatory lesions. Therapeutic approaches to HS may be medical, surgical and/or instrumental. Among the medical therapies, antibiotics are frequently used to treat HS but few data on their efficacy are available. The aim of the present study was to assess the efficacy and the tolerability of a 10-week combination of systemic clindamycin (600 mg daily) and rifampicin (600 mg daily) in the treatment of patients with severe HS. Clindamycin and rifampicin are antimicrobial agents that inhibit bacterial protein synthesis, but their effects is supposed to be both anti-bacterial and anti-inflammatory. The authors have compared the results of their study with the literature data. Twenty patients affected by severe HS during active inflammatory phase were enrolled in a prospective non comparative study. The main parameters used to evaluated the efficacy of the treatment have been Sartorius score, assessing the severity of the disease before and after treatment, the number of exacerbations during the three months of treatment compared with those occurring in the previous three months, PGA (patient global assessment) and IGA (investigator global assessment) after treatment. After 10-weeks of treatment the Sartorius score significantly improved and the number of exacerbations was reduced in 19 patients. The two drugs were well tolerated except in one patient who experienced nausea and vomiting. The antibiotic combination of clindamycin and rifampicin resulted effective in the treatment of severe HS and well tolerated. The same as it can be seen in literature. In this study patients were not randomized in a treatment group versus a placebo group for ethical reasons. This combination was not compared with other treatments because in author's experience it is the best option in severe HS in term of efficacy, tolerability, quick onset of action and the cost.

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Very low electromagnetic field and innate immunity in HaCaT cells

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Defensins (DF) comprises a large group of host defence peptides. In recent years, the functions of DF as immunomodulatory and tissue repair agents have been widely investigated. Several evidences exists that electromagnetic fields (EMF) can influence both inflammatory processes andrepair mechanisms on different tissue models. To study the effect of extremely low frequency EMF (ELF-EMF) on Anti Microbial Peptides components of the human innate immune system, human keratinocyte cell line (HaCaT) was exposed at 1 mT, 50 Hz and compared with unexposed cells at different time points (2, 18, 36 hours). Cytokines and DF gene expression profile were analyzed by polymerase chain reaction. Our preliminary results show that IL-1β and IL-10 cytokine expression were upregulate after 2 hours of ELF-EMF exposure. However while IL-1β expression was not significantly influenced by 18 and 36 hours of continuous exposure, IL-10 expression was increased after 18h and returned to normal levels after 36 hours. Interestingly human beta defensin (HBD) 2 and 3 are unregulated after 2h of ELF-EMF exposure. Meanwhile, only HBD-2 was sensibly decreased after 18h, with no significant variation observed at 36h. No differences were observed in HBD-3 expression at 18 and 36h. Simultaneously ELF-EMF does not seem to influence Vitamin D Receptor (VDR) expression in all investigated time points. These preliminary results may show that ELF-EMF modulates cytokines expression in HaCaT cells. The upregulation of IL-1β could be responsible for the induction of HBD-2 and HBD-3. This could explain how ELF-EMF acts on tissue repair, at least in part, enhancing DF expression through a different pathway than VDR activation. More experiments are needed to better elucidate the actual molecular mechanism that induces the relative immunosuppression and tissue repair enhancement determined by ELF-EMF

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Oral Supplementation of Omega-3 as an adjunct in the Treatment of Psoriasis

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One of the mechanisms that induce inflammation in psoriasis depends on cycloxygenase 2 enzyme activity. This enzyme is produced during the inflammatory response and acts on polyunsaturated fatty acids, leading to formation of cell signaling molecules, like prostaglandins, thromboxanes, leukotrienes. When cycloxygenase 2 acts on arachidonic acid, which is an omega-6 fatty acid, it produces prostaglandins E2, with pro inflammatory and proliferative action in most tissues. However, when there is a bigger contribution of omega-3 essential fatty acids, specially eicosapentaenoic acid and docosapentaenoic acid, they become the main substrates of the enzymatic reaction, producing eicosanoids without inflammatory activity. The purpose of this study is to demonstrate the effects of phospholipids rich in omega-3 polyunsaturated fatty acids in psoriasis, through objective analyzes of PASI and BSA reduction. Thirty patients between 28 and 70 years of age were evaluated with psoriasis vulgaris. PASI and BSA were calculated, and photographic register was made at the beginning and after 60 days of treatment using 400 mg daily of phospholipids rich in omega-3 polyunsaturated fatty acids. Mean reduction in PASI was 52%. It was expressed, specially, by the reduction of the erythema and the infiltration. The BSA reduction was less pronounced (41%). In addition, there was important reduction of the subjective symptoms reported by patients, mainly itching. This study shows that omega-3 polyunsaturated fatty acids administration, reducing the proporcion of omega-6/omega-3, can divert the enzymatic activity of cycloxygenase 2 for the main degradation of omega-3 fatty acids, producing eicosanoids without inflammatory activity and, consequently, reducing the inflammatory manifestations. It is assumed that this is only an adjunct therapeutic modality, with the purpose of adding benefits to the already established treatments of the disease.

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Clinical features and course of Pemphigus

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Many factors affect the clinical course of pemphigus. At this study, we aimed to investigate the effects of factors other than the ones reported at literature, such as delay at diagnosis, compliance to follow-ups, treatment protocols, site of lesion and site of lesion resistant to treatment, on clinical course of pemphigus. A total of 61 pemphigus patients (mean age=53.03±13.86; F/M=1.2/1; follow-up duration =37.7±32.2 months) who were followed at our clinics between the years 2005 and 2010, diagnosed by DIF, IIF and ELISA methods besides histopathological evaluation. A positive correlation was found between the decrease of the PDAI at the beginning and last visit of the treatment and compliance to follow-ups (r=0.53, p=0.01). It was observed that at patients who had an oral lesion as the onset lesion, recurrences and treatment-resistant lesions occurred mostly at mouth (p=0.01). At patients with PF, it was detected that patients who had onset lesions on their bodies had resistant lesions on their bodies as well (p=0.02). It was demonstrated that intralesional CS treatment added to CS treatment provided a decrease at VAS and oral PDAI scores, compared to CS treatment alone (p=0.03, p=0.03). It was observed that treatment-related side effects were more frequent at CS treatment. Besides osteoporosis was related to duration of CS use (p=0.01). It was observed that familial history of DM and high BMI increased the risk fo treatment-related DM (p=0.03, (p=0.04). It was considered that compliance to follow-ups may positively affect clinical course. It must be considered that treatment-resistant lesions and recurrences occur mostly at onset site of disease. Intralesional CS may be recommended additional to systemical CS, especially at treatment of oral lesions. The relation of osteoporosis development with duration of CS use, and DM development with familial DM history and BMI should be warning for detection and prevention of side effects.

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Psychological factors in chronic urticaria – preliminary results

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Chronic urticaria is regarded as psychodermatological condition - stress could cause or exacerbate skin lesions, but also the disease could be a source of stress and exerts negative impact on patients' quality of life (QoL).The aim of the study was to evaluate the relation between psychological factors, itch and chronic urticaria patients QoL. 13 chronic urticaria patients participated in the study (11 females, 2 males; age range: 37-68 years; mean+/-SD 51.62+/-11.26). Duration of the disease ranged from 6 months to 10 years; mean+/-SD 3 years+/-3,21 months. Disease severity was estimated by Visual Analog Scale (VAS) in patients' evaluation. It ranged from 0 to 9.7 (mean+/-SD 4.15+/-3.20). Itch was examined by Itch Severity Evaluation Questionnaire (Szepietowski, Reich, 2010) and ranged from 3 to 15 (mean+/-SD 4.15+/-3.20). The patients completed set of following questionnaires (all validated in Polish): Hospital Anxiety and Depression Scale (HADS, Zigmond, Snaith), Sense of Coherence (SOC-29, Antonovsky), Social Readjustement Rating Scale (SRRS, Holmes, Rahe), Perceived Stress Scale (PSS-10, Cohen, Kamarck, Mermelstein) and Dermatology Life Quality Index (DLQI, Finlay, Khan). Pearson correlation coefficient (r) was assessed and the statistical significance was set at $p<0.05$. The relation between itch and depression score was noted – the more intensive itch, the higher depression level was manifested (r=0.88). The stress level (VAS) correlated negatively with SOC subscale resourcefulness – ones who were more stressed reported lower ability to cope with difficult situations (r=-0.88). Negative association between stress level (VAS) and stressful life events was discovered–patients who declared being more stressed experienced less difficult situations to cope with (r= -0.88). Itch could significantly influence chronic urticaria patients' mood, so it is important to take it into account in all analyses. The results indicated that there could not be a direct connection between stressful life events occurrence and high stress level – coping with stress could be an essential intermediary factor.

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Comparison between TCA peels versus Erbium:YAG Laser in the treatment of Xanthelasma

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Introduction & Objectives: Xanthelasma palpebrarum is one of the cosmetically disturbing lesions affecting periocular region. It affects all age groups but most commonly middle aged females. Many treatment modalities have been used as: CO2 & Er:YAG lasers, surgical removal, chemical substances application as phenol & TCA (trichloroacetic acid). Material & Methods: In this clinical trial, 19 middle aged females suffering from macular & flat xanthelasma palpebrarum were included. Our aim was to evaluate the effect of different TCA concentrations (35%, 50% & 80%) on xanthelasma & compare it with the results of Er:YAG laser. The patients were divided into 4 groups, three groups with 5 patients each. The 1st group was treated with TCA 35%, 2nd group was treated with TCA 50% and the 3rd group with TCA 80%. The 4th group consists of 4 patients treated with Er:Yag laser. Results: The 1st group showed mild unsatisfactory results following 3 sessions with 2 weeks spacing between sessions, a fourth session with TCA 50% was applied to 1st group after which complete disappearance of lesions occurred. The 2nd group showed partial improvement after 2 sessions with complete disappearance of lesions after 3rd session. The 3rd group had great results from the 1st session and only 2 patients out of 4 patients needed a 2nd session with TCA 80%. The 4th group showed complete cure after 2 sessions of Er:YAG. The most common complication of TCA application was mild inflammation which subsided in less than 18 hours after the session. Another problem which occurred in 6 patients was the black colored scales which was formed after 30 hours & totally removed after about 72 hours. Only one patient of the TCA 80% group experienced hypopigmentation. On the other hand, the entire 4th group experienced a painful burning sensation durig the session & reddness & slight scaling shortly after the sessions. Conclusions: Finally, I think TCA 50% & 80% are effective & cheap treatment of flat & macular xanthelasma. ER:YAG Lasers are also effective but very expensive & associated also with the risk of recurrence.

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Indigo naturalis up-regulates claudin-1 expression in cultured human keratinocytes and psoriatic lesions

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The use of indigo naturalis to treat psoriasis has proved effective in our previous clinical studies. To investigate the effect of indigo naturalis on claudin-1 expression in cultured human keratinocytes and psoriatic lesions. The expression of claudin-1 was analyzed in cultured primary human keratinocytes (PHK) by fluorescent immunostaining, real-time RT-PCR and Western blot analysis. The effect of indigo naturalis on tight junction (TJ) function was measured by transepithelial electrical resistance (TEER) and paracellular tracer flux. Claudin-1 expression in psoriatic plaque with or without indigo naturalis treatment was analyzed by immunohistochemical method. Effects of indigo, indirubin, and tryptanthrin, three major components identified in indigo naturalis, on the TJ function were also studied. Indigo naturalis, at non-cytotoxic concentrations, enhanced the expression of claudin-1 in cultured PHK which were validated at the mRNA and protein levels with dose-dependence. Indigo naturalis increased activity of protein kinase C (PKC) in PHK; and indigo naturalis-induced claudin-1 expression was inhibited by a PKC inhibitor. The increase of TEER and reduction of permeability of the 4 kDa FITC-dextran in indigo naturalis-treated keratinocytes were further demonstrated. The three major components of indigo naturalis synergistically exerted effects on up-regulating TJ function with indirubin accounting for the majority of the effect. Notably, the restoration of claudin-1 was observed in healed psoriatic lesions after indigo naturalis treatment as compared with the unhealed psoriatic lesions treated by vehicle. Collectively, these data suggest that indigo naturalis could up-regulate claudin-1 expression in cultured PHK and indirubin provide the major contribution. Indigo naturalis may help restore skin TJ function when used in treating psoriasis.

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Ultrasonography reveals nail thickening in patients with chronic plaque psoriasis

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Background: Nail psoriasis is usually investigated and diagnosed by clinical examination. Ultrasonography is a non-invasive imaging technique for studying soft tissue involvement. Objective: Estimating nail involvement in patients with chronic plaque psoriasis by ultrasonography. Methods: Prevalence, clinical type and severity of nail involvement according to nail psoriasis and severity index (NAPSI) was investigated in 138 patients with psoriasis. The thickness of the plate and bed of the fingernails were measured in 54 patients with psoriasis, 46 healthy controls and 37 patients with chronic eczema, using an ultrasonographic system equipped with a frequency transducer of 18 MHz. Results: The prevalence of nail psoriasis was 73% (102 out of 138). Onycholysis and thickening of the nail plate were the most common clinical type affecting 56% and 50% of patients, respectively; splinter haemorrhages was the less common involving 10% of patients. The mean NAPSI score was 18.4 ± 17.5 (SD; range 0-107). The thickness of fingernail plate and bed was significantly higher in patients with psoriasis with nail disease compared to healthy controls and patients with chronic eczema ($p<0.001$). There was a linear correlation between NAPSI and plate and bed nail thickness ($r=0.52$ and $r=0.38$, $p=0.001$). Increased nail plate and bed thickness was observed also in patients with psoriasis without clinically apparent nail involvement. Conclusions: Thickening of the nail is a common feature of nail psoriasis also in patients without clinically apparent nail involvement.

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Xanthoma Disseminatum, a rare non–Langerhans cell histiocytic syndrome

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This work raises questions about monitoring and treatment of xanthoma disseminatum (XD), a rare non–Langerhans cell histiocytic syndrome. The non-Langerhans Cell Histiocytoses (non- LCH) is a disease entity presenting lesions in various organs as well as diffuse skin lesions. XD typically involves the skin, particularly the flexor skin folds and eyelids. Clinically it can be divided into three groups, those that predominantly affect skin, those that affect skin but have a major systemic component, and those that primarily involve extracutaneous sites, although skin may be involved. The disease often begins insidiously but may lead to extensive morbidity and mortality. No familial cases have been reported while the disease is more prevalent among males. This chronic disease has not known established treatment. XD is a rare non–Langerhans cell histiocytic syndrome that predominantly affects young men and seems to be sporadic. The symptomatic lesions may be cured by surgery, the role of radiotherapy and chemotherapy is unconfirmed. We present the case of a 43-year-old white man, in excellent health status, who had in our department during the period of last two years twenty nine periorbital and three at the area of trunk atypical xanthogranulomas excised. Lastly he appeared with diffuse red or brownish papules and nodules on his armpits and back. The biopsy specimen revealed non–Langerhans cell histiocytosis confirmed by immunohistochemical studies. On XD clinical presentation depends at different stages of maturation of the precursor cell. Meticulous follow up of the patient is mandatory in order to promptly control skin damage and to reveal future involvement of extracutaneus sites. Radiotherapy, Chemotherapeutic agents and steroids have a role in palliating local symptoms but their objective response to the control of disease stabilization is unconfirmed since the response to any form of therapy has been unsatisfactory.

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Antibiotic susceptibility of genital Ureaplasma urealyticum and Mycoplasma hominis

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To determine antibiotic susceptibility of female genital U.urealyticum and M.hominis in the Centre of Dermatovenereology in 2010. To analyse retrospectively all female visits to the Centre for STI in 2010 and whom the cultures for U.urealyticum and M.hominis were performed. Antimicrobial susceptibility to macrolides, clindamycin, tetracyclines, fluoroquinolones was determined. 1058 females visited the Centre for STI in 2010. Out of 122 cultures 61 were positive: 53 -for U.urealyticum, 7 -for both bacteria, 1 -only for M.hominis. U.urealyticum susceptibility to erythromycin, clarithromycin, azithromycin, clindamycin, doxycycline, tetracycline, pefloxacin, ofloxacin, minocycline was 88.7%, 49.1%, 92.5%, 81.1%, 88,7%, 83%, 83%, 41.5%, 86.8% respectively. Mixed infection cultures showed the highest resistance to azithromycin (85.7%), clarithromycin (85.7%), erithromycin (71.4%), the highest susceptibility (71.4%) -to clindamycin, doxycycline, tetracycline. Kendall's Tau-b coefficients showed significant relations between resistance to azithromycin and doxycycline, erythromycin and clindamycin in cultures of U.urealyticum. The resistance to pefloxacin presumed resistance to tetracycline. In most cases U.urealyticum was detected as single pathogen most susceptible to azithromycin, while erythromycin, doxycycline could be a 2nd choice drugs. Clarithromycin, ofloxacin were least effective. In mixed infection the highest susceptibility was to clindamycin, doxycycline, tetracycline. In cultures of U.urealyticum relations were found between resistance to azithromycin and doxycycline, erythromycin and clindamycin. Resistance to pefloxacin is related to the resistance to tetracycline.

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A Novel Fluorescent Assay Used to Measure the Absorption Efficacy of a Sonic Applicator

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Eye serums/creams are typically applied to the delicate skin around the eye area using a gentle tapping motion of the ring finger. A sonic applicator was invented to replace manual application by generating gentle sonic pulses that rapidly (but gently) massages serums/eye creams into outer most layers of the stratum corneum. In order to determine the efficacy of this technology a novel fluorescent serum-dye assay was developed to assess the efficacy of sonic application. A single visit study was conducted to assessed absorption by different application methods. In this study a non-invasive fluorescent serum-dye method, as well as photographs, and image analysis were utilized. A fluorescent marker (cosmetic ingredient oxyresveratrol) was mixed into an anti-aging sea serum (final mixture 0.1% oxyresveratrol) and applied to the lower eye area, both manually and with the sonic applicator. The same volume of the serum-dye mixture (≈50µl) was applied to lower area of both eyes. Participants were instructed to apply the serum-dye mixture to the right lower eye area using their ring finger as they normally would at home, and to the left lower eye area using the sonic applicator. Immediately after application, the serum-dye mixture was blotted twice with an absorbent tissue in a consistent and systematic way to remove excess serum remaining on the surface of the skin. Photographs were taken using a Visia CR camera. Ultraviolet images revealed a disproportional amount of fluorescent marker remaining on the sonically applied eye area. The fluorescence remaining around the eye area after application was quantified by using image analysis software [Image J 1.43; NIH]. Results were then compared statistically using a paired t-test. A statistically significant difference ($p=0.003$) was detected between the manual and sonic application. Photographs and Image J analysis confirm that sonic application using a fluorescent marker results in more absorption into the outer most layers of the stratum corneum than manual application.

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Evaluation of a Novel Sonic Brush Head to Deeply Cleanse Pores

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The purpose of this study was to evaluate the efficacy of a newly designed deep pore (DP) cleansing brush head in conjunction with a handheld battery operated sonic skin care brush. The convex surfaces formed from the nasal folds of the nose often have large deep pores and are considered areas on the face that are difficult to cleanse. Using a newly developed fluorescent microsphere assay the pores on the sides of the nose were used to assess the efficacy of the DP cleansing sonic brush head A single visit, split nose (n=20), randomized study was conducted using a non-invasive fluorescent microsphere assay, photographs, and image analyses. In the fluorescent microsphere assay a mixture of black polyethylene (27 - 45μm) and fluorescent yellow (20 – 27 μm) microspheres were used to label the pores. A 1 to 1mixture of microspheres was applied on the left and right sides of the nose in such a manner as to fill the pores with microspheres. Any loosely adhered microspheres were removed using damp gauze. A randomization schedule identified the cleansing method per side of the nose. Each side of the nose was cleansed simultaneously with the identified cleansing method and a soap/water mixture. After cleansing the remaining soap/water mixture was removed using damp gauze. Photographs were taken before and after cleansing. The fluorescent microspheres remaining in the hard to reach areas (creases) of the nose after cleansing were quantified by using image analysis software [Image J 1.43; NIH]. The side of the nose cleansed with the DP cleansing brush head had significantly less fluorescence in pores (955 pixels) compared to manual cleansing (3410 pixels). Results were compared statistically using a paired t-test. A statistically significant difference (p=0.0004) was detected in deep pore cleansing efficacy between the new sonic brush head and manual cleansing. Analysis confirmed that the new sonic brush head is superior in deep pore cleansing and removed significantly more microspheres from hard to reach areas of the nose (creases) than manual cleansing.

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Tattoos in the emotional perspective

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Nowadays tattoos become more and more popular. By people tattooing their body, they are considered to be not only a cosmetic skin adornment but also the way of one's emotional expression. The aim of the study was to evaluate different emotional aspects (emotional intelligence, control of emotions such as anger, anxiety and depression) and sense of self-efficacy in people with tattoos. The research group consisted of 32 people with tattoos (14 females, 18 males; age range: 18-40 years; mean+/-SD 26.84+/-5.69; tattoo number range: 1-24; mean+/-SD 5.68+/-6.73). Every patient completed set of psychological questionnaires: Emotional Intelligence Questionnaire INTE (Schutte, Malouff, Hall, et al; Polish adaptation Ciechanowicz, Jaworowska, Matczak), Courtauld Emotional Control Scale (CECS, Watson, Greer; Polish adaptation Juczynski), The General Self-Efficacy Scale(GSES, Schwarzer, Jerusalem; Polish adaptation Juczynski). Pearson correlation coefficient (r) was assessed and the statistical significance was set at $p<0.05$. Most respondents (78.1%) reported that their motivation to do tattoo was their personality expression.There were found no statistically significant difference in emotional intelligence, emotions' control and sense of self efficacy depending on respondents' sex, age and tattoo number. There was observed connection between general emotional intelligence (r=0.75), its elements (ability to recognize emotions (r=0.62), ability to regulate emotions (r=0.51) and ability to use emotions to support thinking and performing (r=0.31)) and self-efficacy. The conducted study indicated the relation between emotional intelligence and self-efficacy (treated as the personal resource to cope with stress), which could be the proof of emotional intelligence significance in adaptive functioning. It seems to be also interesting to extend the research by comparing obtained results with control group consisting of people without tattoos.

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Evaluation of the effects of anticellulite therapy with the use of radio frequency (RF) monitored by high-frequency ultrasound

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Cellulite, defined as tissue fibrosis, is a cosmetic defect that affects more than 80% of women and, unfortunately, is unaccepted by most of them which negatively affects their quality of life. Given the size of the phenomenon we are continuously looking for effective ways to reduce cellulite. Reliable monitoring of anti-cellulite treatment remains a problem. The purpose of this study was to evaluate the effectiveness of cellulite treatments carried using tripolar radio waves monitored by means of high frequency ultrasound. 39 women, age 23-52 years, were diagnosed with cellulite. All women underwent 10 treatments with the use of RF. The course of therapy was monitored by high-frequency tests. Ultrasound with the mechanical head of frequency 50 MHz was used. Ultrasound studies examined the following parameters: thickness of the epidermis, dermis thickness, echogenicity of the dermis, the length and surface area of bands of subcutaneous tissue growth in the dermis. Thigh circumference, weight and cellulite stage were also rated (Nürnberger-Müller scale and photonumeric cellulite severity scale) As a result of treatment cellulite reduction occurred in 84.56% of women. Statistically significant decrease in length and area of subcutaneous tissue bands growing into the dermis and increase of echogenicity of the dermis were observed, which is the evidence for the increase in the number of collagen fibers. Cellulite reduction was also confirmed by palpation examination. In conclusion, radiofrequency enables cellulite reduction. A useful aspect is proper monitoring of the progress of such therapy, which high-frequency ultrasound allows.

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Treatment of Lentigo Seniles Using Q-switched Ruby Laser and Q-switched Nd:YAG Laser

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We treated lentigo seniles using Q-switched ruby laser in combination with Q-switched Nd:YAG laser for better therapeutic outcomes. Post inflammatory hyperpigmentation occured after Q-switched ruby laser irradiation for lentigo seniles, was clearly reduced when we irradiated several times with Q-switched Nd:YAG laser with a wavelength of 1064nm and the energy fluence 2.0 -2.5J/cm2, the pulse width of 5-20ns, 6mm spot size, and repetition rate of 5-10 Hz there after. On the other hand, irradiation with Q-switched Nd:YAG laser with the same power on the lesions of the face entirely as a pretreatment reduced the risk of post inflammatory hyperpigmentation after spotty irradiation with Q-switched ruby laser for lentigo seniles. There was no scarring, textual changes or hypopigmentation. The treatment of lentigo seniles using Q-switched ruby laser in combination with Q-switched Nd:YAG laser was found to be excellent therapy.

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Long-term Efficacy and Safety of a Novel Sonic Brush Head to Deeply Cleanse Difficult Areas of the Face

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A new deep pore cleansing brush head using sonic technology was developed to cleanse difficult to reach areas of the face (deep pores, around the nose, base of wrinkles). Long-term efficacy and safety of the deep pore cleansing brush head was evaluated on a population who were currently using sonic technology for facial cleansing. Efficacy was evaluated in an 8 week, 3-visit home use study (n=55) using questionnaire data & before/after use photographs. Safety/gentleness was evaluated in a second randomized study (n=10) using an exfoliation tanning methodology on the lower leg. Transepidermal Water Loss (TEWL), Melanin (M), Skin Moisture/Hydration (H) & Erythema (E) were assessed with the tanning method; before/after 1 minute exaggerated use in a 2″x2″ area for each of the 3 test products [sonic skin care brush using a normal brush head, deep pore cleansing brush head, a nylon facial cleansing pad], as well as a non-treatment control. Duplicate measurements of M, H, E & triplicate measurements of TEWL were taken before/after treatment After 8 weeks of home use 88% of subjects preferred the new deep pore cleansing brush head to the sonic brush head they were previously using, & perceived the deep pore cleansing brush head cleansed better overall (81%), improved the clarity of their skin (81%), & provided a deep pore cleansing (93%). Parametric statistical methods were used to evaluate the safety/gentleness. TEWL measurements after product use fell within the normal range previously established for skin on the lower leg. The average TEWL for the face & lower leg are similar. Little change in M or E was observed following excessive use. Final post treatment measurements of H were higher than untreated control for both the normal & deep pore cleansing sonic brush heads. There was a statistically significantly increase in H post treatment from the control (p=0.02) for the deep pore cleansing brush head. Analysis confirmed the new deep pore cleansing brush head is safe/gentle for daily use & cleanses difficult to reach areas of the face.

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Mozart's Music Effect on Dehydroepiandrosterone-Sulphate in Dermatological in-Patients

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The purpose of this study was to evaluate the effect of Mozart music on selected physiological and psychological stress parameters in skin diseased in-patients. In dermatological in-patients, physiological and psychological sets of measurements were performed twice (before Mozart listening and after). Saliva samples measuring Dehydroepiandrosterone-Sulphate (DHEA-S) levels (ELISA) were collected. In total, six samples were taken from each patient, before Mozart listening and after. Samples were collected at three different time points during three days (morning, afternoon, evening). Furthermore, psychological questionnaires measuring mood (University of Wales Mood Adjective Checklist), depression (Beck's Depression Inventory), stress (Rahe and Holmes Scale), and behavior (Formal Characteristics of Behavior – Temperament Inventory) were employed. Based on saliva measurements, a strong tendency was found between the last measurements of both groups (before and after Mozart; p= 0.054), and a statistically significant difference between the first and last measurement of the Mozart group (p=0.021) was found. Additionally, patients presented weaker depression symptoms after listening to Mozart (p= 0.089; strong tendency). Furthermore, we observed increased saliva DHEA-S levels after three experimental days. In conclusion, listening to Mozart might be beneficial for chronic skin patients, and might be useful as adjunct to pharmacotherapy.

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Assessing efficacy and safety of a novel sonic applicator after long term home use

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A new sonic applicator (SA) to apply eye serums/creams to the delicate under eye area simulating the gentle tapping motion of the ring finger has been invented. The SA generates gentle sonic pulses that rapidly (but gently) applies eye serums/creams to outer most layers of the stratum corneum. The purpose of the study was to clinically assess efficacy & safety via measurements of skin hydration, elasticity, user perception (questionnaire), & before/after use photographs over 12 wks. A 3-visit, randomized (anti-aging sea serum, Pro RT3 serum) study with women between the age of 35 - 60 (n=55) with visible signs of aging was conducted. At baseline, the study examiner used the SA to apply the randomized serum to the left under eye area for 30 seconds. Subjects used the SA to apply the serum to the right under eye area for 30 seconds. Skin hydration [(H) Corneometer] was measured at baseline (before & 15 minutes after product use). Skin elasticity [(E) Cutometer] was measured at the baseline, 4 & 12 week study visits. Photographs & completion of a questionnaire at each study visit were obtained to assess efficacy & safety. Subjects were instructed to use the SA/study serum at home twice/day (morning/bedtime). Results for each time point were compared statistically using a paired t-test. A statistically significant increase ($p<0.001$) in H was measured after 15 minutes of use for both serums & majority of subjects perceived their skin was more hydrated in areas where the serum was applied (95%: anti-aging sea serum; 90%: Pro RT3 serum). After 12 weeks a statistically significant increase ($p<0.01$) in both gross & net E was measured after use of the SA with both the serums. Majority of subjects perceived their skin had more elasticity or firmness in areas where the serum was applied with the SA (84%: anti-aging sea serum; 88%: Pro RT3 serum). Safety results were confirmed by a separated study conducted by an independent Dermatologist and Ophthalmologist. The study confirmed the SA was clinically effective and safe to apply eye serums/creams to the delicate under area